B. Paludis: A New Species of Pathogenic Anærobic Bacterium

BY

A. D. McEWEN, B.SC., M.R.C.V.S.

(FROM THE RESEARCH INSTITUTE IN ANIMAL PATHOLOGY, ROYAL VETERINARY COLLEGE, LONDON).

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(From the Research Institute in Animal Pathology, Royal Veterinary College, London.)

The bacterium which is described in this paper has been encountered on several occasions during investigations on diseases of sheep on the Romney Marsh, Kent. The particular diseases are known locally by the terms "struck" and "gangrene." The term "struck" is applied by the farming community to a rapid and fatal disease where post-mortem examination reveals an acute inflammatory condition in one or more of the following parts of the body: areas of muscular tissue, organs in the abdominal cavity, and organs in the thoracic cavity. The term "gangrene" applies to a similar disease in ewes attacked within a few days after lambing.

From the investigations carried out during the past two years, evidence shows that a number of species of pathogenic sporulating anærobic bacteria may each separately be responsible for these diseases.

The particular bacterium now under consideration is one of the number of species encountered, and is described separately because it is of peculiar interest, as it has been recovered from the muscular tissue where the lesions were in many ways similar to those of black-quarter, and because the bacteria resemble *B. welchii*, and may readily be mistaken for that micro-organism unless careful and detailed bacteriological examinations are made.

The bacteria probably belong to a hitherto unrecognised species, and if this be so, I suggest that the bacterium should be named *B. paludis*; this name is proposed because the bacteria have been found causing disease in animals grazing and living on a marsh.

Material From which *B. paludis* was Isolated.

In each instance the material consisted of muscular tissue which was forwarded to the laboratory in sterile containers.

Given below, for each case, are the descriptions of the lesions in the sheep as tabulated by the owner ; the appearance of the specimen when received at the laboratory, together with the particulars of microscopical examinations of smears made from the muscle ; the preliminary cultural findings ; and the results of guinea-pig inoculations.

Specimen No. 14.

This consisted of a portion of muscle, dull red in colour, soft, and friable. Particulars of the post-mortem examination were not received. Smears showed numerous moderately large, stout, stumpy bacilli with bluntly rounded ends ; no spores were seen ; the bacilli were Gram-positive.

Cultures showed that ærobic contaminating bacteria were present, and pure cultures of *B. paludis* were not obtained by direct cultural means. A guinea-pig inoculated subcutaneously with 0·1 c.c. of a mixed broth culture died, and from its heart blood *B. paludis* was isolated.

Specimen No. 29.

The owner reported that the subcutaneous tissue, the muscular tissue, and the stomach and intestines were affected. When the muscle was received it was deep red in colour, and obviously diseased. Smears showed an apparently pure and rich culture of non-sporulating bacilli similar to those seen in smears from Specimen No. 14. *B. paludis* was recovered in pure culture from the muscle ; no other bacteria were encountered. 0·1 c.c. of a broth culture inoculated subcutaneously killed a guinea-pig in less than 24 hours, and the same bacillus was recovered from the animal's heart blood.

Specimen No. 32.

This portion of muscle was received at the laboratory two days after the animal's death. The report stated that the subcutaneous tissue, the muscular tissue, the stomach, the intestines, and the lungs were affected. The appearance of the muscle and the microscopical and cultural examinations were the same as those reported for Specimen No. 29, and 0·1 c.c. of broth culture inoculated subcutaneously proved lethal for a guinea-pig.

Specimen No. 33.

The muscle was received at the laboratory two days after the animal's death. The owner reported that he had not seen the sheep from which the specimen came, but his shepherd had said that no obviously diseased areas were seen in the carcase. Unfortunately, records of the appearance of the specimen when it was received at the laboratory were not kept. Cultures revealed ærobic contaminating bacteria, and *B. paludis* was not recovered by direct cultural means, but the bacillus was isolated from the heart blood of a guinea-pig inoculated with 1 c.c. of a saline emulsion of the muscular tissue and found dead on the morning following inoculation.

Specimen No. 34.

This specimen was received four days after the animal's death. The owner reported that the subcutaneous tissues, the muscular tissues, the stomach, the intestines, and the lungs were all affected. When the portion

of muscle was received, it was dark red in colour, moist, and inflamed ; a sourish smell was noted. Smears showed Gram-positive bacilli similar to those met with in Specimen No. 14, and *B. paludis* was recovered from the specimen in pure culture, no other bacteria being present.

Specimen No. 38.

This was received at the laboratory two days after the sheep had been found dead. The owner reported that lesions were present in the subcutaneous and muscular tissues of the neck and back, and that the stomach and intestines and lungs were affected. Upon receipt the muscle appeared red in colour, but apart from that there was no obvious abnormality. Smears showed bacilli similar to those from Specimen No. 14, and *B. paludis* was recovered in pure culture, no other bacteria being present. 0·1 c.c. of broth culture inoculated subcutaneously into a guinea-pig resulted in the animal being found dead 17 hours later. Cultures from the heart blood of this guinea-pig remained sterile.

Specimen No. 40.

This came from a sheep that had died three days previous to the receipt of the specimen. The owner reported that the subcutaneous and muscular tissues were affected. The muscle was moister than normal and deep salmon-pink in colour. Smears revealed a picture similar to that described for smears from Specimen No. 14. Cultures showed that *B. paludis* was not present in a pure state, but pure cultures were eventually obtained by direct cultural methods, and 0·1 c.c. of a broth culture proved lethal in 20 hours for an inoculated guinea-pig. Cultures from the heart blood of this animal showed bacilli similar to those used in the inoculation.

Specimen No. 47.

The specimen was received at the laboratory the day following the animal's death. The shepherd reported that lesions were present in the musculature of the throat and breast, and that the lungs were affected. The portion of muscle received was normal in appearance, but smears showed bacilli similar to those described from Specimen No. 14. *B. paludis* and *V. septique* were recovered by direct cultural methods. They appeared to be the only bacteria present. 0·1 c.c. of a broth culture of the *B. paludis* strain was inoculated subcutaneously into a guinea-pig, and the animal was found dead 18 hours later. The heart blood of this guinea-pig was sown into appropriate media, but no growth was obtained.

Specimen No. 49.

The sample of muscle was received at the laboratory the day after the animal's death. The owner reported that lesions were present under the skin. The muscle and the associated fat appeared deep pink in colour and excessively moist. The specimen had a slightly unpleasant and sourish smell. Smears showed numerous bacilli similar to those described from Specimen No. 14. By direct cultural means pure cultures of *B. paludis* and *V. septique* were obtained. A third type of colony growth was also observed, but pure cultures of the bacillus producing these colonies were not recovered by direct cultivation. A mixed culture, however, in 0·5 c.c. amount was inoculated subcutaneously into a guinea-pig. This animal died with a typical *B. chauvœi* infection, and *B. chauvœi* was isolated from its heart blood.

Specimen No. 50.

A portion of muscle was received at the laboratory two days after the animal's death. It was deep pink in colour and softer and more friable than normal. Smears presented a similar picture to those obtained from Specimen No. 14. *B. paludis* was recovered from the muscle in what appeared to be pure culture, and 0·5 c.c. was inoculated subcutaneously into a guinea-pig. The animal died in 48 hours, and *B. paludis* and *V. septique* were both recovered from its heart blood. 0·1 c.c. of a pure culture of the *B. paludis* strain isolated from the guinea-pig was inoculated into a second guinea-pig, and this animal was found dead the following day, and *B. paludis* was recovered from its heart blood in pure culture.

A guinea-pig inoculated subcutaneously with 1 c.c. of a normal saline emulsion of the sheep's muscle died in 24 hours. Post-mortem examination failed to show any sign of *V. septique* infection, but the lesions and the examination of smears made from the peritoneal fluid indicated that death was due to *B. paludis*. Culture media inoculated with the heart blood of this guinea-pig remained sterile.

Specimen No. 52.

This consisted of two pieces of muscle which came from a ewe that had lambed three weeks previously. The specimens were received the day after the animal was found dead. Both pieces of muscle were deep pink in colour, but one more so than the other. Smears were made from both pieces of muscle. Those from the dark coloured portion failed to show any bacteria, but smears from the remaining portion showed large numbers of bacilli similar to those described from Specimen No. 14, and cultures from this portion of muscle gave a pure growth of *B. paludis*. 0·1 c.c. of a broth culture was inoculated subcutaneously into a guinea-pig, and the animal was found dead 18 hours later. Cultures from the heart blood of this guinea-pig were not made, but it is of interest to note that a guinea-pig inoculated with 1 c.c. of a saline emulsion of the sheep's muscle died ; the post-mortem examination revealed a typical *B. paludis* infection, but cultures from the animal's heart blood remained sterile.

Specimen No. 75.

No particulars were received with this specimen, which consisted of a portion of muscle, œdematous and hæmorrhagic in appearance and of a sour offensive odour. Smears showed a large number of bacilli, similar to those present in smears from Specimen No. 14, but a larger sporulating bacillus was also present though in much smaller numbers. Cultures showed that a variety of ærobic contaminating bacteria were present, and pure cultures of *B. paludis* were not obtained by direct cultural means. A colony of *B. paludis* type, however, was sown into a tube of broth minced meat medium, and 0·2 c.c. of the resulting growth was inoculated subcutaneously into a guinea-pig. The animal died, and from its heart blood *B. paludis* was recovered.

From the above descriptions it will be seen that in the majority of cases the muscular tissue was abnormal in appearance and obviously diseased. In many cases *B. paludis* was the only micro-organism present, though in others it has been found associated with *V. septique*, and once apparently with *V. septique* and *B. chauvœi*, but in these cases an examination of smears made from the muscular tissues failed to reveal any evidence of infection with either *V. septique* or *B. chauvœi*.

When ærobic bacteria were present they were regarded as extraneous contaminating bacteria. In the case of Specimen No. 75 a large sporulating type of anærobic bacterium was present in the muscle. This was not isolated, but the specimen was not fresh, and in all probability the sporulating bacillus was a post-mortem invader.

The great number of Gram-positive, thick, stumpy, and relatively large bacilli which were present in all the specimens were identical in their appearance with the vegetative forms of *B. welchii*, and presented a picture similar to that found in smears made from the lesions in the muscular tissue of a sheep caused by *B. welchii* infection. (McEwen, 1930).

The Pathogenicity of *B. paludis*.

The pathogenicity of the majority of the strains was tested by inoculating guinea-pigs subcutaneously with small quantities of broth culture. No attempt was made to ascertain the minimum lethal dose, but when 0·1 c.c. of culture was inoculated it invariably proved fatal. Amounts of less than 0·1 c.c. were not tested.

The lesions shown in animals succumbing to inoculation of culture consisted of an extensive necrosis of the subcutaneous and muscular tissues. The skin over the affected parts became moist through the exudation of fluid, and the hair was easily detached. In the subcutaneous tissues there was a quantity of liquid material which could be detected as a fluctuating mass before incision of the skin was made. This liquid material was of a dirty greyish-pink colour, turbid in appearance, and contained fragments of necrotic tissue. The underlying muscles were pale and extensively necrosed and autolysed, and frequently a loop of intestine protruded through a hernia in the necrotic abdominal wall. The subcutaneous tissues of parts more distant from the seat of inoculation, *e.g.*, the axillæ and the under surface of the neck, were infiltrated with a pink, coagulated, gelatinous material from which, on cutting, fluid exuded. The abdominal cavi y presented a picture of generalised congestion, the blood vessels being markedly injected. The stomach, intestines, and adrenals were congested. Smears made from the surface of the peritoneum showed vegetative forms of *B. paludis*, ranging from single rods to short chains of four individual bacilli. Long chains were never seen. The number of bacteria in peritoneal smears varied greatly from case to case. In animals with a hernia of the abdominal muscles the bacteria were present in great numbers, but in other cases they were comparatively scanty, only one or two being found in each field. Cultures from the heart blood were in some instances positive, but in others no growth occurred.

B. paludis, on one occasion only, has been inoculated into a sheep. The particular sheep used was hyperimmunised to *V. septique* infection and resisted large inoculations of that micro-organism without showing any ill effects. 0·5 c.c. of a broth culture of *B. paludis* was inoculated intramuscularly into the thigh. Fifteen hours later the animal was very lame, and it died at the forty-second hour. The inoculated leg

was greatly swollen from the thigh to the hoof. The skin on the inside of the thigh in the neighbourhood of the seat of the inoculation was purple in colour. The subcutaneous tissues of the leg and of the inguinal region were infiltrated and distended with a pale yellow, and in parts slightly blood-tinted, gelatinous material from which on incision fluid in large quantities escaped. Over the leg this infiltration reached a thickness of 2 to 5 cm., but in the region of the groin it was even greater The vessels in the subcutaneous tissues were injected and conspicuous. The musculature of the inoculated leg was pale, except for some reddened areas in the thigh, and sections through these showed that the red discoloration was due to intramuscular hæmorrhages situated around the more peripheral parts of the muscles. The intramuscular septæ were infiltrated with material similar to that encountered in the subcutaneous tissues. On opening the abdominal cavity the omentum presented an appearance of patchy inflammation due to congestion of the small blood vessels. The abomasum showed acute inflammatory areas in the mucosa, particularly in the neighbourhood of the greater curvature. The first few inches of the small intestine were acutely congested, but the remainder appeared to be normal. The large intestine was congested. The spleen presented numerous small subcapsular hæmorrhages. The liver, kidneys, and adrenals appeared to be normal. The thoracic cavity and the organs therein appeared normal. Smears were made from the fluid in the subcutaneous tissues and from the surface of the peritoneum, but no bacteria were found in them. Smears from the muscle of the inoculated thigh showed a few bacilli only. Cultures were made from the heart blood into suitable media, but these remained sterile.

The evidence which has been summarised above shows *B. paludis* to be a pathogenic bacterium capable of producing a fatal disease of sheep in the field, which disease may be reproduced experimentally in sheep by the inoculation of cultures of the bacilli. The disease is characterised by an acute inflammatory reaction in parts of the muscular and subcutaneous tissues of the body, the bacteria being present in these areas and generally in great numbers. The stomach and intestines may be involved and show congestion of their walls. *B. paludis* is commonly the only micro-organism present in the esions in the muscular tissue, but it has also been found in association with other pathogenic anærobic bacteria. Microscopical examinat ons of smears made from the muscular tissue, however, have not indicated the presence of these other pathogenic bacteria, and the evidence points to *B. paludis* as being the primary cause of death in all of the twelve cases here reported.

Morphological and Cultural Characters of *B. paludis*.

The twelve strains of *B. paludis* considered in this paper were all obtained in pure culture before details of their characters were considered. Pure cultures were secured by repeatedly subculturing, alternately on serum agar slopes and in broth containing minced meat.

Individual colonies on serum agar were sown each into a separate tube of broth minced meat medium, and growth from this medium was in turn seeded on to serum agar. The process was repeated until the purity of the strain was satisfactorily established.

Morphology.

Bacilli from 24 hours' broth minced meat medium appeared as simple vegetative rods, single or in pairs. The bacilli were often collected into small bundles, the individual bacilli being in parallel formation. The bacilli were stout and stumpy, with bluntly rounded or almost square cut ends, in shape and size closely resembling the well-known *B. welchii*. Spores were not seen in smears made from cultures in this medium ; but from alkaline egg medium and cultures on solid serum sporulating forms were observed, the spores being oval and of greater width than the bacillus, and either central or subterminal in position. Citron or spindle-shaped forms of the bacteria were not noticed.

The bacilli stained intensely with ordinary aniline dyes and the great majority in young broth minced meat cultures were strongly Gram-positive, though a number showed irregularities in their retention of the stain, the commonest being an unstained area running the length of the bacillus. A few bacilli were entirely Gram-negative.

Bacilli from broth minced meat cultures were examined for the presence of a capsule, but capsule formation was not demonstrated. Bacilli, however, from animal tissue and fluids were not submitted to this examination, and the question of capsule formation has yet to be definitely settled.

Motility.

Sixteen and twenty-hours' broth minced meat cultures were tested for motility by placing a small quantity of the culture on a slide, covering with a coverslip, ringing round the edge of the cover slip with warm liquid vaseline, and examining under the No. 6 objective. Examined in this manner, the great mass of the bacilli were totally without motion, but occasionally bacilli appeared to perform slow tumbling and turning movements, and to be sluggishly motile. The slight degree of motility exhibited was too indefinite to be of any critical value, and was such that independent observers differed among themselves in their interpretation as to whether motility was present or absent.

Examinations of *B. paludis* under dark ground illumination have not revealed the presence of flagella, which suggests that the sluggish motility of certain individuals observed when the No. 6 objective was used was not due to true bacterial motility but to some other factor.

Cultural Characters.

In all instances cultures were maintained at a temperature of 37° C.

The bacilli were anærobic ; no growth occurred either in liquid or on solid media under ærobic conditions.

The McIntosh and Fildes electrically warmed anærobic jar was used to secure anærobiasis for surface growths, and in the case of liquid media free O_2 was excluded by covering the surface of the liquid with a layer of vaseline before sterilisation of the media. All inoculations of liquid media were made with a Pasteur pipette through the vaseline seal previously liquefied by warming. The volume of the gas evolved in liquid media was estimated roughly by noting the extent of the upward displacement of the vaseline seal.

Serum agar. Surface colonies varied considerably in size. The smaller colonies were 1 to 2 m.m. in diameter. These were simple round, convex, smooth colonies, greyish-white in colour, opaque, with regular free margins, and raised upon the surface of the medium. Larger colonies up to 5 or 6 m.m. across had a similar colour and consistency. They were, however, more spreading in character and relatively flatter, and in many instances their free border was irregular, presenting blunt indentations, and running from each indentation towards the centre of the colony low ridges were sometimes observed under the hand lens ; these ridges faded out before the centre of the colony was reached. The most central part of the colony was either flat or slightly raised.

Blood agar. The colonies were similar to those descr bed on serum agar except that on this medium they appeared less opaque and were surrounded by a wide zone of hæmolysis.

Shake agar. Deep colonies were opaque and lenticular and up to 2 m.m. in diameter.

Shake blood agar. Deep colonies were similar to those produced in shake agar, and were surrounded by a clear zone of *beta* hæmo.ysis.

Broth containing minced meat. Vigorous growth occurred at the end of 24 hours, with the evolution of gas and the production of a moderate turbidity of the medium. At three days there was an increased pink coloration of the meat, and after one week the meat was changed to a deep pink colour, and there was considerable disintegration of the meat particles, but no distinct digestion. Incubation for periods of four to five weeks produced no further change. Blackening of the meat or the formation of black pigment under the vaseline seal was never noted.

Broth. After 24 hours there was evolution of gas and the medium was uniformly turbid. On the third day the turbidity showed signs of clearing, and at the end of one week the medium was clear, and a small white compact deposit had settled out in the bottom o the tube.

Broth containing a small portion of brain tissue and a piece of iron wire. After a week's incubation in this medium blackening was observed under the vaseline seal and on the sides of the tube beneath the seal and above the level of the broth. The brain tissue sometimes showed traces of blackening. No further change was produced by longer incubation.

Skim milk. At the end of 24 hours there was vigorous gas production, and a large greatly fragmented clot was formed (typical "stormy" clot reaction). At the end of three days the clot was reduced in size, and after one week a spongy shrunken clot remained, reduced to approximately one-third of its original size. The expressed whey was clear. At the end of four weeks of incubation the culture assumed a faint buffy tint, but no further change was noted.

Skim milk containing a small portion of brain tissue. The growth in this medium was similar to that in skim milk.

Cream (1 *c.c. of washed, sterilised cream added to* 10 *c.c. of broth*). After three days' incubation there was no appreciable alteration in the appearance of the cream, but after one week's incubation the cream was converted to a flocculent greyish-white material. No further alteration was noted at the end of four or five weeks' incubation.

Alkaline egg medium. This medium was rendered turbid, but no coagulum or large flocculi were produced.

12½ *Gelatine in broth.* When cultures were removed from the incubator they were placed in the ice chest for an appropriate period, and then examined for liquefaction. Uninoculated tubes were treated in a like manner and acted as controls. Gelatine was liquefied in 24 hours.

Coagulated egg white cubes in broth. No alterations were noted in the character of the egg white until incubation had been continued for three to four weeks, when a darkening in colour and an increased transparency of the cubes were produced. At the end of five weeks' incubation the cubes were of a clear deep brown colour towards their centres, while the edges were clear and transparent.

Inspissated serum slopes. After four weeks' growth slight erosion of the serum at the lower part of the slopes was noted. Incubation for a period of eight weeks produced distinct but not extensive liquefaction of the medium with accumulation of fluid.

Fibrin. (*Small piece of fibrin in a tube of broth.*) No evidence of any digestion or alteration of the fibrin was observed even after prolonged incubation.

Fermentation reactions, acid and gas production being taken as indications of fermentation. The basal medium consisted of peptone water containing a small cube of brain tissue. The materials to be tested were sterilised separately by autoclaving, and a sufficient quantity of a 10 per cent. solution was added to the basal medium to make the final concentration of the test substance 1 per cent. The *p*H of the finished medium was remarkably constant for each batch produced, but different batches of media varied in their *p*H from 6·5 to 7·0. Fermentation was considered to have occurred when the *p*H of the medium was reduced by 0·5 or more and when a volume of gas at least equal to half the volume of he culture fluid was produced. The *p*H of the medium was read after one week's incubation and again after four weeks' incubation by the colorimetric method, small quantities of fluid (0·5 c.c.) being tested and matched against buffer solutions of known *p*H. The two readings were very

constant for all the substances tested except salicin, which in some instances gave a markedly lower *p*H reading at the second test.

Glucose, lactose, levulose, galactose, maltose, saccharose, and glycerine were fermented by all the strains, and with the exception of Strain 32 all the strains fermented salicin. Glycerine and salicin did not give rise to such an abundant volume of gas nor to such an extensive drop in the *p*H as did the fermentable sugars. Inulin and mannite were not fermented. The utilisation by the bacilli of 0·2 per cent. of glucose, lactose, levulose, galactose, maltose, and saccharose in the basal medium, was checked by testing for the presence of reducing sugars in the media after one week's incubation by the Benedict qualitative method, the technique described by Brown (1925) being adopted. In no instance was any sugar detectable after one week's growth, and these results confirm the fermentation reactions recorded for the same sugars when acid and gas productions were the criteria of fermentation. Before applying the Benedict test when saccharose was used the sugar was converted to a reducing sugar by boiling the sample of the medium to be tested with dilute HCl.

Comparison of the Morphology, Cultural Characters, and Virulence of *B. paludis* and *B. welchii*.

Morphology.

In smears stained by ordinary methods both the vegetative and sporulating forms of *B. paludis* and *B. welchii* are very much alike, and it is not possible to distinguish one from the other. No capsule has been demonstrated for *B. paludis*, but absence of capsule cannot at present be taken as a differential character distinguishing the one type of bacillus from the other, because cultures of *B. welchii* were examined for capsule formation under comparable conditions and none was found, although Robertson (1916) states that *B. welchii* is invariably a capsulated bacillus and that the capsule may be seen in cultures examined in weak alkali solution or serum.

The motility of *B. paludis* is very doubtful, but, even assuming true bacterial motility to be present, the degree of motility demonstrable by the technique employed is not sufficient to make it either an easy or reliable method of distinguishing *B. paludis* from the non-motile *B. welchii*.

Cultural Characters.

In their cultural characters *B. paludis* and *B. welchii* closely resemble one another.

The shape and form of colonies, the stormy clotting of milk, the absence of coagulation in alkaline egg medium, and the liquefaction of gelatine in cultures of *B. paludis*, agree with the accepted cultural characters of *B. welchii*. The *beta* type of hæmolysis produced by *B. paludis* and the formation of a flocculent material from cream are in agreement with the reactions of *B. welchii* described by Brown (1925), and the production of black pigment in media containing

iron by *B. paludis* is similar to the reaction produced by *B. welchii* under comparable conditions (Hall, 1922 ; Kahn, 1925). But when the action of the two bacilli over long periods of time upon coagulated egg white and solid serum is considered, differences in behaviour become apparent. *B. paludis* darkens egg white, and causes it to become transparent, and solid serum is slowly liquefied. This bacillus is therefore more proteolytic than *B. welchii*, which does not attack or alter these proteins (Brown, 1925).

The fermentation reactions of the two bacilli show much in common, both fermenting a wide range of substances, including glucose, lactose, levulose, saccharose, galactose, and maltose.

All the twelve strains of *B. paludis* fermented glycerine, but none fermented inulin. On these fermentations of glycerine and inulin *B. paludis* cannot be differentiated from *B. welchii*, as strains of *B. welchii* vary in their capacity to ferment glycerine and inulin, some strains fermenting either the one or the other, while yet other strains ferment both substances. It is, however, agreed that *B. welchii* does not ferment salicin. Salicin was fermented by eleven of the twelve strains of *B. paludis* studied, and in this respect the eleven strains differ from *B. welchii*.

Virulence. According to the majority of authors cited by Robertson (1929) and O'Brien (1929), *B. welchii* is pathogenic for guinea-pigs in amounts of 0·2 c.c. to 1 c.c. inoculated intramuscularly. *B. paludis* has invariably been found pathogenic for guinea-pigs when 0·1 c.c. of a culture of broth minced meat medium has been inoculated subcutaneously. This small amount of culture produced extensive lesions and death in 24 hours or less. Smaller quantities of culture would, in all probability, have proved fatal for guinea-pigs, but these were not tested. Up to the present there has been no appreciable loss of virulence in strains kept in artificial culture for several months. *B. paludis* would therefore appear to be a more virulent and more highly pathogenic bacterium than *B. welchii*, but at present this greater virulence must be regarded as one of degree only, and not as a reliable means of distinguisling *B. paludis* from *B. welchii*, though it may serve as an indication of which micro-organism is being dealt with.

The lesions produced in guinea-pigs by *B. paludis* and *B. welchii* are similar.

Cultures of *B. paludis* have not been inoculated into mice, but small quantities of bacteria-free filtrates have frequently been inoculated into these animals with fatal results. In no instance has hæmoglobinuria ever been observed in mice dying from inoculation with *B. paludis* filtrates, although this particular feature has been observed frequently in mice inoculated with *B. welchii* filtrate, and is a well recognised, though not constant, post-mortem characteristic of this condition. It may therefore be concluded that *B. paludis* intoxication does not give rise to a hæmoglobinuria, and in this respect differs from intoxication with *B. welchii*. Further, the toxin of *B. paludis* is distinct and different from that of *B. welchii*, but this subject is discussed at greater length later on.

Another bacillus which might appear similar to *B. paludis* is the " lamb dysentery bacillus," described by Dalling (1928), but the characters of this bacillus, as described by that author, which distinguish it from *B. welchii* serve equally well to distinguish the " lamb dysentery bacillus " from *B. paludis*, as, for example, the active proteolytic properties of the " lamb dysentery bacillus," which enable it rapidly to liquefy solid serum, its coagulation of alkaline egg medium, and its inability to ferment glycerine, are all features which are not shared by *B. paludis* and should prevent confusion arising between the two species.

B. paludis Toxin.

Cultures were obtained in diffusion shell 1 per cent. glucose broth medium *p*H 7·4 (McEwen, 1926), and after 24 hours' incubation the rich growths were centrifuged until they were clear ; the clear fluids were then filtered through Seitz filters and the filtrates tested for sterility. The toxicity of the sterile filtrates from the twelve strains was tested by the inoculation of laboratory animals.

In all twelve instances small quantities of filtrate inoculated intravenously into rabbits caused death in a few minutes ; and those filtrates which were tested by the subcutaneous inoculation of rabbits and guinea-pigs, or by the intramuscular inoculation of mice, produced an extensive œdema and necrosis and, if sufficient filtrate were inoculated, the death of the animals.

Table No. 1 shows the result of inoculating varying quantities of filtrate intravenously into rabbits.

TABLE I.

Rabbit.	*Weight.*	*Amount of filtrate inoculated intravenously (Filtrate from Srain 52).*	*Result.*
1	2500 grams	2 c.c.	Dead in 3 minutes
2	2200 grams	1 c.c.	Dead in 7 minutes
3	2500 grams	0·5 c.c.	Dead in 7 minutes
4	2150 grams	0·25 c.c.	Died overnight
5	2000 grams	0·125 c.c.	Died overnight
6	1900 grams	0·0625 c.c.	Lived
7	2050 grams	0·03125 c.c.	Lived

TABLE II.

Records the Result of the Intramuscular Inoculation of Mice with Varying Quantities of Filtrate.

Mice.	*Amount of filtrate inoculated intramuscularly (Filtrate from Strain 52).*	*Result.*
1 & 2	0·15 c.c.	Both dead in less than 24 hours
3 & 4	1 c.c.	Both dead in less than 24 hours
5 & 6	0·05 c.c.	One dead in less than 48 hours. The other survived the 48th hour but inoculated leg was swollen and paralysed.
7 & 8	0·02 c.c.	Survived but in both ; the inoculated leg was swollen and paralysed.
9 & 10	0·004 c.c.	One had a slightly swollen leg. The other remained normal.

The Effect of Heat Upon Toxic Filtrate.

Filtrate from Strain 52 was heated in the water bath for periods of 30 and 60 minutes at a temperature of 60° C. Animals inoculated with the heated filtrate remained normal. The results of these inoculations are shown in Table III. The effect of temperatures lower than 60° C. upon toxic filtrate, or of a temperature of 60° C. acting for a period of less than 30 minutes, has not been studied.

TABLE III.

Rabbit.	*Weight.*	*Amount of filtrate inoculated into each animal.*	*Result.*
8	1950 grams	1 c.c. of filtrate heated to 60° C. for 60 minutes, inoculated intravenously	Lived
9	1950 grams	1 c.c. of filtrate heated to 60° C. for 30 minutes, inoculated intravenously	Lived
Mice			
11 & 12		0·15 c.c. of filtrate heated to 60° C. for 60 minutes, inoculated intramuscularly	Remained normal
13 & 14		0·15 c.c. of filtrate heated to 60° C. for 30 minutes, inoculated intramuscularly	Remained normal

The Effect of Storage at Low Temperature upon Toxic Filtrate contained in a hermetically sealed tube and in a tube plugged with porous cotton wool.

Filtrates were stored in the refrigerator at a temperature of approximately 2° C. for eight weeks, but at the end of this period no appreciable diminution in toxicity was detected ; filtrates from the hermetically sealed tube and the tube plugged with cotton wool both killed rabbits a few minutes after intravenous inoculations were made.

Immunisation Against Toxic Filtrates of B. paludis.

Filtrate from Strain 32 in 0·5 c.c. amount inoculated intravenously into a rabbit of 2,500 gms., killed the animal in 10 minutes. The broth culture, from a portion of which this filtrate had been made, was divided into two parts and to one 0·8 per cent. of formalin, and to the other 0·4 per cent. of formalin, was added. The formalinised cultures were then incubated at 37° C. for 48 hours and stored in the refrigerator.

Four rabbits—No. 13, 2,100 gms. ; No. 14, 2,500 gms. ; No. 15, 2,700 gms. ; and No. 16, 2,500 gms., were inoculated intravenously with 1 c.c. of the supernatant fluid collected after centrifuging that portion of culture containing 0·8 per cent. formalin, and two days later they again each received another intravenous inoculation of 3 c.c. No symptoms were produced by the inoculations, and later the immunisation was continued by the intravenous inoculations of the supernatant fluid from the portion of culture treated with 0·4 per cent. formalin. This treatment was finally followed up by the subcutaneous inoculation of small amounts of fresh toxic filtrate without formalin.

At the end of the period of immunisation Rabbit No. 15 was inoculated intravenously with 1·5 c.c. of filtrate 32. This animal

remained well, but a control rabbit, No. 17, of 2,600 gms., inoculated at the same time and with a similar amount of the filtrate, died in four minutes. Rabbits Nos. 13, 14, and 16 were each inoculated subcutaneously with 3 c.c. of filtrate 32. No lesion resulted from the inoculation, but of the control rabbits Nos. 18 and 19, of 2,500 gms. each, which were each inoculated with the same amount of filtrate, No. 18 was found dead the following morning, and post-mortem examination revealed great infiltration of the subcutaneous tissues with a gelatinous, semi-liquid, pale straw-coloured material, and hæmorrhages in the underlying musculature ; while No. 19 was dull, the inoculated leg being greatly swollen and the skin purple in colour. On the second day it was considered necessary to chloroform the animal, and the post-mortem examination showed a picture similar to that described for rabbit No. 18.

From these experiments it is clear that in rabbits Nos. 13, 14, 15, and 16 an active immunity against the toxic filtrates of *B. paludis* had been produced.

Passive Immunity to the Toxic Filtrates of B. paludis.

Rabbits Nos. 13, 14, 15, and 16, after immunisation, were bled, and the serum from each animal was tested for antibodies which would neutralise the toxic action of filtrates. Sera from animals Nos. 14 and 16 appeared to be the more potent, and these sera were pooled, and used in the tests recorded below.

Two guinea-pigs were each inoculated subcutaneously with 3 c.c. of *B. paludis* antiserum. Two days later these and two control guinea-pigs each received a subcutaneous inoculation of 0·5 c.c. of filtrate from Strain No. 29. The day following the inoculation of the filtrate both of the control guinea-pigs had extensive fluctuating œdematous swellings at the seat of the inoculation. One of the guinea-pigs treated with serum showed a small circumscribed œdematous swelling, while the other animal treated with serum appeared normal. On the third day the control guinea-pigs had very extensive œdematous swellings, but the animals which had received serum had the merest trace of swelling at the point where the inoculation of the filtrate was made. On the fifth day the skin over the affected parts of the control animals was moist, necrotic, and denuded of hair, while the serum-treated animals showed small areas $\frac{1}{4}$-in. in diameter covered by a dry scab. The extensive lesions in the control animals necessitated their being chloroformed, but the other animals remained bright, and rapidly lost all evidence of having been inoculated.

The results of the above experiment showed that the inoculation with the immune serum gave the guinea-pigs a distinct protection against the toxic principal in the filtrate.

The Specificity of the Toxic Filtrates of B. paludis.

As a close resemblance exists between *B. paludis* and *B. welchii* it was deemed essential to study the action of *B. welchii* antiserum

upon the toxic filtrate of *B. paludis*. This was particularly necessary as *B. welchii* has been recovered from the musculature of a sheep which presented a similar appearance to the specimens from which *B. paludis* has been isolated (McEwen, 1930). Further, *B. welchii* has been reported by Meissner and Albrecht (1924) and by Karmann and Seifreid (1924) as the occasional cause of black-quarter or gas œdema in sheep, but these authors apparently arrived at a diagnosis without the aid of neutralisation tests of toxin or virulent culture by specific immune serum.

Through the kindness of Mr. J. H. Mason, F.R.C.V.S., of the Wellcome Physiological Laboratories, a supply of *B. welchii* antiserum was received, and parallel tests with mixtures of *B. paludis* filtrate and its specific antiserum were conducted along with mixtures of *B. paludis* filtrate and *B. welchii* antiserum. The mixtures were allowed to stand at room temperature for one hour before the inoculations were made. These tests are recorded in Table No. VI. The toxic filtrates from each of the twelve strains of *B. paludis* were neutralised by the *B. paludis* antiserum, but were not affected by the *B. welchii* antiserum.

At this stage of the work attempts were made to obtain a potent *B. welchii* toxin in the hope of testing the action of *B. paludis* antiserum upon *B. welchii* toxin, but a potent *B. welchii* toxin was not obtained and, accordingly, the possibility of *B. paludis* antiserum neutralising the pathogenic action of cultures of *B. welchii* was investigated, and

TABLE VI.

INTRAMUSCULAR INOCULATION OF MICE.

Mouse No.	*L.D. filtrate.*	B. paludis *antiserum.*	B. welchii *antiserum.*	*Result.*
		Toxic Filtrate No. 32 *L.D.* 0·2 *c.c.*		
15 & 16	0·2 c.c.	—	—	Both dead in 24 hours
17 & 18	0·2 c.c.	0·1 c.c.	—	Lived. No symptoms
19 & 20	0·2 c.c.	0·05 c.c.	—	Swelling of inoculated leg. Both survived
21 & 22	0·2 c.c.	—	0·1 c.c.	Both dead in 24 hours
23 & 24	0·2 c.c.	—	0·05 c.c.	Both dead in 24 hours
		Toxic Filtrate No. 47. *L.D.* 0·15 *c.c.*		
25 & 26	0·15 c.c.	—	—	Both dead in 24 hours
27 & 28	0·15 c.c.	0·1 c.c.	—	Swelling of inoculated leg. Both animals lived
29 & 30	0·15 c.c.	0·02 c.c.	—	Both dead in 24 hours
31 & 32	0·15 c.c.	—	0·2 c.c.	Both dead in 24 hours
33 & 34	0·15 c.c.	plus 0·1 c.c. normal rabbit serum		Both dead in 24 hours
		Toxic filtrate No. 52. *L.D.* 0.15 *c.c.*		
35 & 36	0·15 c.c.	—	—	Both dead in 24 hours
36 & 37	0·15 c.c.	0·1 c.c.	—	Inoculated leg swollen. Both lived
38 & 39	0·15 c.c.	—	0·2 c.c.	Both dead in 24 hours
40 & 41	0·15 c.c.	plus 0·1 c.c. normal rabbit serum		Both dead in 24 hours

TABLE VI.—*Continued.*

INTRAVENOUS INOCULATIONS OF RABBITS.

Rabbit No.	*Weight.*	*L.D. of filtrate.*	B. paludis *antiserum.*	B. welchii *antiserum.*	*Result.*
			Toxic Filtrate No. 52.		
23	2200 gms.	1 c.c.	—	—	Died in 7 minutes
24	2050 gms.	1 c.c.	1 c.c.	—	Lived
25	2500 gms.	1 c.c.	0·5 c.c.	—	Lived some hours. Died over night
26	2200 gms.	1 c.c.	0·25 c.c.	—	Died in 12 minutes
27	2400 gms.	1 c.c.	—	1 c.c.	Died in 3½ minutes
28	2000 gms.	1 c.c.	plus 1 c.c. normal rabbit serum		Died in 3½ minutes
			Toxic Filtrate No. 14.		
29	2200 gms.	0·5 c.c.	—	—	Died in 6 minutes
30	1500 gms.	0·5 c.c.	0·5 c.c.	—	Lived
31	2400 gms.	0·5 c.c.	—	1 c.c.	Died in 4 minutes
			Toxic Filtrate No. 29.		
32	2200 gms.	0·5 c.c.	—	—	Died in 4 minutes
33	2000 gms.	0·5 c.c.	0·5 c.c.	—	Lived
34	2000 gms.	0·5 c.c.	—	1 c.c.	Died in 4 minutes
			Toxic Filtrate No. 33.		
35	2200 gms.	0·5 c.c.	—	—	Died in 10 minutes
36	2050 gms.	0·5 c.c.	0·5 c.c.	—	Lived
37	1500 gms.	0·5 c.c.	—	1 c.c.	Died in 7 minutes
			Toxic Filtrate 34.		
38	1850 gms.	0·25 c.c.	—	—	Died in 8 minutes
39	2200 gms.	0·25 c.c.	0·5 c.c.	—	Lived
40	2500 gms.	0·25 c.c.	—	1 c.c.	Died in 10 minutes
			Toxic Filtrate No. 38.		
41	2300 gms.	0·5 c.c.	—	—	Died in 14 minutes
42	2000 gms.	0·5 c.c.	0·5 c.c.	—	Lived
43	2200 gms.	0·5 c.c.	—	1 c.c.	Died in 30 minutes
			Toxic Filtrate No. 40.		
44	2000 gms.	0·5 c.c.	—	—	Died in 16 minutes
45	2400 gms.	0·5 c.c.	0·5 c.c.	—	Lived
46	1850 gms.	0·5 c.c.	0·5 c.c.	—	Lived
47	2250 gms.	0·5 c.c.	—	1 c.c.	Muscular weakness in 30 minutes, but animal appeared to recover ; died on 3rd day
48	1700 gms.	0·5 c.c.	—	1 c.c.	Died in 20 minutes
			Toxic Filtrate No. 49.		
49	2600 gms.	0·5 c.c.	—	—	Died in 17 minutes
50	1950 gms.	0·5 c.c.	0·5 c.c.	—	Lived
51	2200 gms.	0·5 c.c.	—	1 c.c.	Died in 25 minutes
			Toxic Filtrate No. 50.		
52	2000 gms.	0·5 c.c.	—	—	Died in 6 minutes
53	2800 gms.	0·5 c.c.	0·5 c.c.	—	Lived
54	2300 gms.	0·5 c.c.	—	1 c.c.	Died in 13 minutes
			Toxic Filtrate No. 75.		
55	2700 gms.	0·5 c.c.	—	—	Died in 6 minutes
56	2000 gms.	0·5 c.c.	0·5 c.c.	—	Died in 30 minutes
57	2000 gms.	0·5 c.c.	—	1 c.c.	Died in 6 minutes
58	2000 gms.	0·5 c.c.	0·5 c.c.	—	Lived
59	2300 gms.	0·5 c.c.	—	1 c.c.	Died in 8 minutes

at the same time the capacity of the *B. welchii* antiserum to protect against the pathogenic action of cultures of *B. welchii* was tested.

It was found that *B. paludis* antiserum had no neutralising effect upon the pathogenicity of cultures of *B. welchii*, though the same cultures were rendered harmless for inoculated animals by the action of the *B. welchii* antiserum. These results are recorded in Table No. VII, and the combined results of the experiments enumerated in Tables Nos. VI and VII furnish proof that *B. paludis* is an independent and distinct species from *B. welchii*.

TABLE VII.

THE ACTION OF *B. paludis* ANTISERUM AND OF *B. welchii* ANTISERUM UPON BROTH CULTURES OF B. WELCHII.

Two Strains of B. welchii *were used, one was Strain No. 273 of the National Collection of Type Cultures, and the other was a strain isolated from the muscle of a sheep. The mixtures of serum and cultures were inoculated into the subcutaneous tissues.*

Strain.	*L.D. of culture*	*Guinea pig.*	B. paludis *antiserum.*	B. welchii *antiserum.*	*Result.*
273	0·25 c.c.	1	—	—	Died in 24 hours
273	0·25 c.c.	2	—	—	Died on third day
273	0·25 c.c.	3	0·75 c.c.	—	Died in 24 hours
273	0·25 c.c.	4	0·75 c.c.	—	Severe and extensive lesions. Chloroformed on third day
273	0·25 c.c.	5	—	0·25 c.c.	Remained well
273	0·25 c.c.	6	—	0·25 c.c.	Remained well
273	0·25 c.c.	7	—	0·1 c.c.	Remained well
273	0·25 c.c.	8	—	0·1 c.c.	Remained well
Isolated from a sheep	1 c.c.	9	—	—	Died in 16 hours
,,	1 c.c.	10	—	—	Died in 16 hours
,,	1 c.c.	11	0·75 c.c.	—	Died in 16 hours
,,	1 c.c.	12	0·75 c.c.	—	Died in 16 hours
,,	1 c.c.	13	—	0·5 c.c.	Remained well
,,	1 c.c.	14	—	0·5 c.c.	Remained well
,,	1 c.c.	15	—	0·1 c.c.	Severe local lesions, but recovered
,,	1 c.c.	16	—	0·1 c.c.	Severe local lesions; but recovered

The evidence also points to a distinction between *B. paludis* and the " lamb dysentery bacillus." The " lamb dysentery bacillus " shows in its antigenic complex an antigen common to itself and *B. welchii*, as the antiserum of the former is capable of neutralising the toxin of the latter although the reaction is not a reversible one (Darling, 1928). *B. paludis* antiserum, however, has no such property.

The rapidity of the lethal action of *B. paludis* filtrates when inoculated intravenously into rabbits resembles the action of *V. septique* toxin (Robertson, 1920) and *B. histolyticus* toxin (Weinberg and Seguin, 1917). Confusion of the identity of these micro-organisms should never arise on account of their marked dissimilarity in morphology and cultural reactions. Nevertheless, as *V. septique* had in a number of instances been isolated from material from which

B. paludis was recovered, it was deemed to be of sufficient interest to test the effect of inoculating animals with mixtures of *B. paludis* filtrate and *V. septique* antiserum. The *V. septique* antiserum was prepared by hyperimmunising a sheep with repeated inoculations of formalinised culture, and these were followed by inoculations of virulent culture. The serum from this animal, in appropriate amounts, completely neutralised quantities of *V. septique* toxin which was capable of killing rabbits in a few minutes, but the same serum had no neutralising effect upon the toxic filtrates of *B. paludis*.

The results of these experiments are given in Table No. VIII.

It is of interest to note that the sheep which supplied the *V. septique* antiserum was the same animal which succumbed in 42 hours to the inoculation of 0·5 c.c. of a culture of *B. paludis*.

TABLE VIII.

THE EFFECT OF *V. septique* ANTISERUM UPON *V. septique* TOXIN AND UPON *B. paludis* TOXIC FILTRATES.

Rabbit.	*Weight.*	V. septique *toxin.*	B. paludis *toxic filtrate.*	V. septique *antiserum.*	B. paludis *antiserum.*	*Result.*
60	2050 gms.	1·5 c.c.	—	—	—	Died in 5 minutes
61	2250 gms.	1·5 c.c.	—	1 c.c.	—	Lived
62	2400 gms.	1·5 c.c.	—	1 c.c.	—	Lived
63	2300 gms.	—	1·5 c.c.	1 c.c.	—	Died in 4 minutes
64	3600 gms.	—	1·5 c.c.	1 c.c.	—	Died in 6 minutes
65	1800 gms.	—	1·5 c.c.	—	1 c.c.	Lived several hours, died overnight

Summary of the Features presented by B. paludis *Toxic Filtrates.*

The intravenous inoculation of rabbits with *B. paludis* filtrate caused the death of these animals in a few minutes, and subcutaneous inoculations of filtrate into rabbits and guinea-pigs and the intramuscular inoculation of mice produced an extensive œdema and necrosis of the inoculated parts and the surrounding tissues, and if sufficient filtrate were inoculated the death of the animals.

Lysis of the red blood cells of inoculated animals has not been observed, and hæmolysis did not result from the incubation of mixtures of washed red blood cells in saline and toxic filtrate.

Rabbits immunised against the toxic filtrates showed a complete resistance to intravenous and subcutaneous inoculations of lethal doses of filtrate, and guinea-pigs inoculated with the serum of immune rabbits have exhibited a markedly increased resistance to the toxic action of subcutaneous inoculations of filtrate.

The toxicity of filtrates was completely neutralised by the action of immune serum *in vitro*.

The evidence concerning the action of formalin upon toxic filtrates, though incomplete, points to the formation of an atoxic antigenic substance.

The toxic principal in the filtrate was thermolabile.

From the foregoing characters exhibited by the toxic filtrates it is concluded that the toxic principle in the filtrates is a true bacterial toxin.

Toxins were easily obtained and have been prepared from strains kept in artificial cultivation for periods of from one to fourteen months. Further, the *p*H of the medium, within the limits which were tested, had little appreciable effect upon the production of toxin, toxic filtrates being obtained from media with a *p*H of 6·8, 7·2, 7·4 and 7·6.

The facility with which *B. paludis* toxin is obtained in filtrates is in contrast with the difficulty so frequently experienced in obtaining toxic filtrates of *B. welchii* (Robertson, 1929).

Symptoms and Post-mortem Appearance shown by Animals Inoculated with B. paludis *Toxin.*

The first rabbit to be inoculated intravenously with toxin received a large dose, between 4 c.c. and 5 c.c. The intention had been to test the effect of the inoculation of 5 c.c., but before the total amount had been inoculated the animal collapsed and died, it being a matter of seconds only from the commencement of the inoculation to the time of death. Smaller quantities of toxin cause death after a variable period ranging from a few minutes to several hours, depending upon the quantity of toxin inoculated. When death occurs a few minutes after inoculation symptoms of muscular weakness are first seen. The ears droop backwards and the head is gradually lowered upon the neck, the muscles of the limbs become flaccid, and the animal lies limply on the ground. During this time the respirations are shallow and more rapid than normal. Death may supervene quietly and peacefully, but more generally it is preceded by a short period of muscular excitability, irregular running or kicking movements being performed as the animal lies on the ground, then for a few seconds respiration cases, and the thorax remains distended, but immediately prior to death one or two gasping respiratory movements are performed. A few fæces are passed at the commencement of the period of muscular weakness. When the appearance of the muscular weakness is delayed for 10 to 20 minutes or longer the symptoms are not so severe ; the animals assume a crouching position and are unable or unwilling to move. Partial recovery takes place after the lapse of a short period, and then, except perhaps for a general dullness in looks and behaviour, the animals appear to be normal. However, they almost invariably die at the end of a few hours. Occasionally symptoms of muscular weakness are not shown, but the animals are found dead several hours after having been inoculated.

The symptoms produced by subcutaneous or intramuscular inoculations of toxin are those associated with the local œdema and

necrosis, but when an excess of toxin is inoculated symptoms of profound intoxication are shown, and death follows.

Post-mortem examinations of animals dying from intravenous inoculation have failed to reveal any noteworthy lesions. In animals inoculated subcutaneously or intramuscularly the lesions of œdema and necrosis only are present, and these are confined to the inoculated and adjacent parts.

General Discussion on the Recognition of *B. paludis*.

To distinguish *B. paludis* from *B. welchii* the most critical test, namely, the neutralisation of toxin or of the pathogenicity of culture by specific immune serum, should be employed. Now, in the identification of *B. welchii* great reliance has been placed upon morphology and cultural characters and serum neutralisation tests have been neglected in the majority of cases, and, when the close similarity of the morphology and cultural reactions of *B. paludis* and *B. welchii* are considered, it is reasonable to suppose that had *B. paludis* been encountered it might have been labelled *B. welchii*. Only within recent years has confusion regarding the identity of *B. chauvœi* and *V. septique* ceased to reign, and the distinguishing features of these two micro-organisms are much more marked and more easily detected than are the characters which distinguish *B. paludis* from *B. welchii*.

Considerable attention has been given to diseases of cattle due to the anærobic sporulating bacteria, but it is doubtful if *B. welchii* by itself produces disease in these animals. Noller and Seelemann (1924) consider that it may do so, but the evidence they bring forward is by no means conclusive. *B. welchii* has on occasion been isolated along with other pathogenic anærobic bacteria from material derived from cases of black-quarter—Warringholz and Rassfeld (1924), Zwick (1924), Scott (1928), Weinberg and Nichailesco (1928). A primary infection of cattle with *B. welchii*, if it does occur, appears to be of but little importance, and hence it is very unlikely that *B. paludis*, which so closely resembles *B. welchii*, is of any importance as a cause of disease in these animals.

Sheep, more than any of the other domestic animals, show a peculiar susceptibility to infection with a variety of the pathogenic anærobic bacteria. Thus, *V. septique* is incriminated in the diseases " bradsot " and " braxy " in Europe, the *B. œdematiens* in the " black disease " of sheep in Australia and New Zealand, *B. chauvœi* as the cause of " black-quarter " of sheep in Europe and America, and the " *lamb dysentery bacillus* " as the cause of disease of lambs in this country. *B. welchii* has been accused as being responsible for disease in sheep in comparatively few instances, and the relative frequency of *B. paludis* and *B. welchii* infections in these animals and the extent to which they occur must remain an open question until the results of carefully conducted investigations become available.

SUMMARY.

From twelve cases of disease in sheep in which the muscular tissue presented lesions characteristic of those produced by the pathogenic sporulating anærobic bacteria, a new species of pathogenic sporulating anærobic bacillus has been isolated. This bacillus has been named *B. paludis*.

In five of the cases *B. paludis* was present in the infected muscular tissue in pure culture, and in all twelve cases smears from the muscular tissue showed the bacillus to be present in very large numbers. *B. paludis* is considered to have been the cause of the disease which brought about the death of the animals.

B. paludis presents many features in common with *B. welchii*. The chief feature distinguishing between the two bacilli is the specificity of their toxins.

Descriptions of the material from which *B. paludis* was isolated, the morphological and cultural characters of the bacillus, its pathogenic action, and its toxin, together with the differentiation of the bacilius from *B. welchii*, have been given in detail.

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"STRUCK": ENTERITIS AND PERITONITIS OF SHEEP CAUSED BY A BACTERIAL TOXIN DERIVED FROM THE ALIMENTARY CANAL.

By A. D. McEwen and R. S. Roberts.

South-Eastern Agricultural College, Wye, Kent.

The Romney Marsh, Kent, is remarkable for the good quality of its pastures and for the large number of sheep they support. The practice of grazing the pasture very heavily has prevailed for years, and it is not surprising that diseases, comparatively unknown in the surrounding country, should be all too familiar on the Romney Marsh. Locally these diseases are recognised as being of two distinct types, and are termed "gangrene" or "ganger" and "struck."

The terms "gangrene" and "ganger" are appropriate and refer to a wound gas gangrene, which may affect the ewe a day or two after lambing, or the lamb after castration or docking; again, the disease occasionally appears in a flock of sheep soon after shearing. There is, however, always a clear history of a wound infection.

The term "struck" is applied to a disease characterised by a short period of illness which terminates fatally and is not associated with a wound infection.

Concerning the incidence of "struck" and the conditions under which it occurs, there is general agreement among owners and shepherds on the Marsh. The disease is said to occur during the late winter and spring, March being regarded as the time when it is most prevalent.

Certain pastures are notorious, while upon others the disease seldom occurs. The incidence of the disease seems to be intimately related to the amount of grazing available for the animals, and the losses from stock are believed to be heaviest when the pastures are short and the grazing is comparatively scanty, but in the absence of exact figures it is impossible to judge the toll exacted by this disease. Five to ten, or even fifteen, per cent. of the animals may be lost in one season on one particular portion of land, while on another the losses may be negligible.

The consensus of opinion is that "struck" is in some manner a dietetic disease in the sense that it is acquired through ingestion of certain pastures, but there is no suspicion that the disease is due to a plant poisoning, and from the fine quality of the pasture the incrimination of noxious weeds or plants seems out of the question.

The symptoms shown by the "struck" sheep are probably overlooked until grave lesions have developed, when the animals are noticed neither to feed nor to ruminate, and appear dull and dejected. Sheep may stand in a strained position, which has given rise to the erroneous opinion that the animals are straining to micturate. This position is probably assumed in an effort to relieve abdominal pain.

Diarrhœa is never alluded to in connection with " struck," nor is coughing, inco-ordination of movement, or cerebral symptoms.

The general knowledge regarding the post-mortem picture is very limited. The descriptions conveyed the impression that post-mortem appearances differed considerably, and that at times advanced changes were present in the organs of the abdominal cavity, while at others the musculature was affected and occasionally even the lungs. But it is common knowledge that if the sheep is detected before death and slaughtered the eviscerated carcase may be dressed for the butcher and sold for human consumption.

Generally no attempt is made to dispose of the carcases in a hygienic manner. The practice is for the shepherd to bring the carcase to a convenient point near his dwelling, skin it, leaving the skin out to dry, and feed the flesh to the dogs. Often no special effort is made to prevent sheep grazing on ground where carcases have lain, but there has been no suggestion that sheep on such ground were any more liable to contract the disease.

Twenty-five years ago Cave (1905) reported the conclusions arrived at after several years of study of the disease, and stated that " struck " and black-quarter were one and the same disease, caused by the same micro-organism, the black-quarter bacillus. Numbers and details of post-mortem and bacteriological examinations were not given in this communication nor in any of his Reports (Cave, 1903, 1904, 1907, 1909), and it is impossible to tell whether he ever worked with material obtained immediately after, or a short time after, the animal's death.

In the Report of the Departmental Committee on Louping-ill and Braxy (1906), the authors refer to the disease " struck " on the Romney Marsh, and say that : " the disease is caused by an anærobe closely allied to that of Braxy and Louping-ill and which, like them, is probably intestinal in habitat." Mention is also made of the view that " struck " is the same disease as black-quarter, but this view is not accepted, although no reasons are given for holding a different opinion other than the bald statement quoted above, that the bacillus is probably intestinal in habitat.

From enquiries made in 1928, it was at first thought probable that the diseases " struck " and " gangrene " were instances of gas gangrene infections and similar in nature, and arrangements were made to have specimens of what appeared to be affected muscles of animals which had died from these diseases forwarded to the Research Institute in Animal Pathology, Royal Veterinary College, London, where they would be examined for pathogenic bacteria.

These investigations proved of value and led to the isolation of strains of a new species of pathogenic sporulating anærobe, *B. paludis*, which appeared to play an important rôle in the ætiology of the disease " struck " (McEwen, 1930). This bacterium and the *V. septique* and *B. chauvœi* were the pathogenic bacteria frequently encountered in the investigations.

The results of the work conducted in London are given below in tabular form. In the Table 52 specimens are accounted for. This does not represent all the specimens received, which actually numbered 81. Of the 29 not included in the Table, three gave pathogenic anærobic bacteria, and one of these was probably *B. paludis*, but this strain was accidently thrown away before the typing was completed ; the other two were bacteria of the *B. œdematiens* type. In the case of the remaining 26 specimens failure in a number of instances to demonstrate pathogenic bacteria was experienced, but the majority were not examined as they were obviously in an advanced state of decomposition.

In many cases specimens were received with no history thereon ; these must be considered as coming from either a " struck " sheep or a sheep which had died of gas gangrene, but from which it is impossible to tell.

When more than one type of pathogenic anærobe were encountered in a specimen only that which appeared to predominate is included in the Table given below.

SUMMARY OF RESULTS OF BACTERIOLOGICAL INVESTIGATIONS SHOWING THE PRINCIPAL TYPES OF PATHOGENIC SPORULATING ANŒROBIC BACTERIA ISOLATED FROM 52 SPECIMENS DERIVED FROM SHEEP SAID TO HAVE DIED FROM " STRUCK," POST-PARTURIENT GAS GANGRENE, OR WOUND GAS GANGRENE.

	No. of specimens from which B. chauœvi *was isolated.*	*No. of specimens from which* V. septique *was isolated.*	*No. of specimens from which* B. paludis *was isolated.*
Specimens with a definite history of " struck "	0	2	6
Specimens from ewes with a history of post-parturient gas gangrene	2	3	0
Specimens from sheep with no definite history	10	18	6
Specimens from lambs with history of having recently been sheared or castrated ...	5	0	0

In a case of apparent *B. paludis* infection which is not included in the Table the history was one of " struck."

From many of these 52 specimens the particular bacterium isolated was present in large numbers to the exclusion of all other demonstrable ærobic or anærobic micro-organisms, and it was necessary when attempting to account for their presence to allow for their being ante-mortem invaders and possibly the cause of the animal's death. This is particularly true when the bacterium isolated is a comparatively fastidious micro-organism like *B. chauvœi*. It may not hold to the same degree for a vigorously spreading and easily recoverable micro-organism such as *V. septique*. In the particular cases from which *B. paludis* were isolated the striking picture presented by the specimens

made it impossible to think that this bacterium was a mere post-mortem invader and casually dismiss it as such.

1930 *Investigations on " Struck."*

The absence of reliable data on the pathology of the disease made it essential that cases should be investigated by post-mortem and bacteriological examinations. Post-mortem examinations have now been made at the time of the animal's death and up to 24 hours after death. A consideration of these examinations has shown that " struck " is a specific disease. But, as was to be expected, animals were sometimes said to be affected when they were suffering from or had died from a totally different disease.

When cultures were made from the tissues or body fluids these were invariably inoculated into broth containing minced meat and under a vaseline seal, and with rare exceptions the material was also inoculated into liver broth and on to liver agar or serum agar. These broth and agar tubes were incubated ærobically and anærobically. From the examination of the primary cultures information regarding the presence of one or more species of bacterium or the absence of bacteria from the seed material was obtained. Liberal quantities of seed material were used, and when body fluids were inoculated into a liquid medium quantities of 1 c.c. or more were sown into each tube.

Whenever *B. paludis* was recovered or was isolated from any particular source the identification of the bacterium in every instance is founded upon cultural characters, the production of *B. paludis* toxin by the particular strain in question, and the neutralisation of the toxin by specific antitoxic serum. The toxin neutralisation test was accepted as the real criterion of identification of the strain (McEwen, 1930).

The Macroscopic Lesions found in Cases of " Struck " where the post-mortem examination was made at the time of death and shortly after death.

Examinations of 17 cases have been made, and these showed remarkably constant lesions. These lesions are regarded as characteristic and consist of an enteritis, an acute peritonitis, and changes attributable to a toxæmia.

The peritoneal cavity contains an excessive quantity of fluid, varying in amount up to three litres or more. The fluid is pale yellow in colour, and lightly turbid, and a short time after death it becomes faintly blood-tinged. Yellow coagula floating in the fluid or adherent to the peritoneum are frequently found. Upon exposure to the air the fluid rapidly coagulates.

The peritoneal vessels are intensely injected and conspicuous, and multiple subperitoneal hæmorrhages may be present. Generally the injection of the vessels is most obvious over the omentum, the course of the small intestine, and the urinary bladder.

The mucous membrane of the abomasum occasionally shows

areas of mild congestion. The small intestine presents changes ranging from a moderate to a most acute congestion of part or parts of its length, and this is frequently accompanied by ulceration of the mucous membrane. The ulcers are generally present in the jejunum, and vary from small areas 2 or 3 mm. in diameter to extensive lesions 6 to 12 cm. in length by 1 to 2 cm. width. These ulcers may be numerous and involve many feet of bowel. The periphery of the ulcers is dark red and the floors are either deep red in colour or covered by a dark greenish adherent deposit. The ulcers rarely have been found extending down to the peritoneal covering of the intestine.

The contents of the small intestine are sometimes noticeably blood stained.

The cæcum and colon are frequently contracted, firm to the touch, with their lumen partially or almost completely obliterated and the mucous membrane thrown into multiple corrugations. When this condition is found fæcal material is absent or very scanty.

Individual mesenteric glands occasionally show a very moderate degree of congestion.

The kidneys sometimes appear to be in a state of cloudy swelling in cases examined at the time of death, the cut surface of the organ being irregular, glistening though dark in colour, and appearing to bulge outwards.

The urine and the urinary bladder have invariably been found normal. Hæmoglobinuria has never been observed.

On a few occasions congested areas have been found in the adrenals.

The liver and spleen appear healthy.

The thoracic cavity generally contains an excessive transudate of a clear yellow colour, and a similar fluid is often present in the pericardial sac.

Subepicardial and subendocardial hæmorrhages may be found in the heart.

The subcutaneous and intermuscular tissues are almost invariably normal, but a moderate infiltration of the abdominal intermuscular septæ with a pale semi-liquid material has been encountered in two instances. Lesions in the muscles suggestive of gas gangrene are not found.

Bacteriological Examination of 17 *Cases of "Struck."*

Material from 17 cases was examined by cultural and other means. In 13 instances *B. paludis* was isolated. In two cases *V. septique* was recovered, once along with *B. paludis* and once accompanied by non-pathogenic anærobic bacteria.

In the majority of cases where *B. paludis* was recovered the bacteria appeared to be widely distributed throughout the body, being recoverable from the heart blood and other organs. However, the bacteria were seldom numerous, except in cases where animals had been dead a few hours and microscopical examination of smears

of tissues and body fluids was frequently negative. Bacteria were most readily found in smears made from the peritoneal fluid. Bacteria have been recoverd from the peritoneal fluid when media seeded with heart blood and tissue from other organs has remained sterile. Failure to cultivate *B. paludis* from the body musculature has been experienced when the bacteria were recoverable from internal organs.

When *B. paludis* was isolated it was generally present in pure culture, but in one instance it was recovered from the peritoneal fluid along with *V. septique* though cultures from the thoracic fluid and spleen gave growths of *B. paludis* only. The only other case where *B. paludis* was not pure was when it was accompanied by a coliform bacterium in the peritoneal fluid.

From three most typical cases no bacteria were recovered. These cases were examined at the time of death, and in each instance the heart blood, peritoneal fluid, and spleen tissue were used for cultivation. In addition, in one instance the thoracic and pericardial fluids were similarly used, in another the thoracic fluid and material from a mesenteric lymph gland, and in the third case material from a mesenteric lymph gland.

B. paludis gives an abundant and vigorous growth, and had it been present there is little doubt but that it would have been demonstrated. The conclusion is therefore reached that *B. paludis* was not generalised throughout the bodies of these three sheep.

Ulcers were present, however, in the small intestine of these animals, and it is possible that bacteria associated with these ulcers by the production of toxin caused a toxæmia. But this explanation is not satisfactory because the disease may occur without there being ulceration, and in the absence of evidence of localised areas of bacterial activity the extensive lesions cannot be satisfactorily attributed to the scanty number of bacteria which may be found throughout the body.

The Histological Examinations of the Small Intestine in Cases of "Struck."

As the small intestine was the organ which showed the most definite inflammatory reaction, sections were prepared from parts macroscopically normal in appearance and from congested and ulcerated areas.

Material was obtained from five cases within a few minutes after death. This showed that the primary lesion consisted of a necrosis of the superficial parts of the mucous membrane. The necrosis progressively extends downwards to the submucous coat and is accompanied by a leucocytic infiltration of the still recognisable mucosa, together with congestion of the vessels and hæmorrhages therefrom.

In the early stage there is no evidence of bacteria in the mucosa, but as the extent of the necrosis increases bacteria may appear in the necrotic tissue, apparently invading it from the lumen of the bowel.

Sections of ulcers occasionally show masses of Gram-positive bacteria morphologically similar to *B. paludis* invading the deeper tissues at the periphery of the ulcer and giving rise to extensive collections of leucocytes in the invaded tissues. Ulcerated areas, however, may show no evidence of such active bacterial invasion of the tissues.

Post-mortem and Bacteriological Examinations Conducted more than eight hours after death.

A number of the cases examined more than eight hours after death were in a very advanced state of post-mortem decomposition and no reliance could be placed on data obtained from them. These cases, therefore, were not examined bacteriologically, and they are not considered here. However, in 13 examinations conducted eight or more hours after death post-mortem changes were less advanced and bacteriological examinations were made.

The most striking changes found were in the subcutaneous, intermuscular, and muscular tissues. The subcutaneous and intermuscular areolar tissues showed various degrees of infiltration with a red, semi-fluid, gelatinous exudate, while the muscles themselves were moist, extremely soft and frequently discoloured in patches, the colour varying from a dirty salmon-pink to a reddish-black. In some instances, the muscles were emphysematous and riddled by gas. The whole picture was very suggestive of acute gas gangrene infection, and the gross appearance comparable with black-quarter.

Another notable feature was the large accumulations of turbid, blood-stained fluid in the abdominal cavity. A similar fluid was sometimes present in the thoracic cavity. In some instances inflammatory reactions were detectable in the alimentary tract, and ulceration of the small intestine has been observed.

Of the 13 cases included in this group, 11 showed the characteristic changes reminiscent of gas gangrene in the subcutaneous areolar tissues and muscles, in nine cases there was excess of fluid in the peritoneal cavity, in four instances some excess of fluid was found in the thoracic cavity, and in four cases evidence of inflammatory changes in the intestines was found.

Smears made from the abdominal fluid showed that more than one species of bacterium was present. However, smears made from the musculature revealed in ten cases a bacillus, morphologically similar to *B. paludis*, in great abundance and apparently uncontaminated. In another case the same bacillus was present in a state of purity in the smears made from the spleen. From seven of these cases *B. paludis* was recovered in pure culture, and from one case *V. septique* was also isolated, although it could only have been present in the tissues in small numbers, and its presence was not suspected by smear examination. In the remaining five cases in which bacilli resembling *B. paludis* were found in smears difficulty was encountered in obtaining pure cultures, and the information obtainable from these did not seem to justify the time which would

have been necessary to purify them. From one of these mixed cultures *V. septique* was readily obtained in a pure state.

Smears from the remaining two cases showed moderately large bacteria, not of the *B. paludis* type. From one of these *V. septique* was isolated, and from the other an unknown anærobic bacterium of low pathogenicity.

From the bacteriological examinations it appeared that *B. paludis* was the bacterium principally involved and responsible for the extensive changes encountered in the subcutaneous, intermuscular, and muscular tissues. The outstanding feature of the post-mortem examinations was the changes in these tissues, and it was at first difficult to reconcile them with the absence of lesions in the muscles and subcutaneous tissues at or soon after death ; but, as *B. paludis* was the micro-organism which could be held responsible in the majority of cases, there was little reason to doubt that the changes which had formerly given rise to the conception that this disease was analogous to black-quarter were of post-mortem generation. The possibility of this was proved by experiment.

The Production of the Changes in the Musculature which Simulate Black-quarter.

The peritoneal fluid of a field case of " struck " (Sheep W. 42) which was obtained six hours after death contained very large numbers of *B. paludis* and was shown to be rich in *B. paludis* toxin.

1.—Approximately 150 c.c. of the unfiltered peritoneal fluid from Sheep W. 42 were inoculated intravenously into a normal sheep. The animal collapsed immediately the inoculation was made, and was dead within six minutes. The post-mortem examination was purposely delayed until 15 hours later, the carcase being allowed to lie in the field, the weather at the time being cool. The subcutaneous tissues contained an abundant accumulation of dark red, soft, gelatinous material and similarly tinted fluid. This was most plentiful in the regions of the neck, axillæ, and back. Similar infiltrations were present in the intermuscular septa. The muscles had a dirty salmon-pink colour, with here and there parts of a deeper and darker tint ; they were moist, very soft and friable, and in places riddled with gas. The subcutaneous and muscular tissues presented a picture exactly comparable to that so frequently met with in cases in the field when the post-mortem examination was made more than eight hours after death. The remaining organs showed putrefactive changes only. No specific lesions were noted in the abomasum or intestines, and no excess of fluid was present in the abdominal cavity.

Smears made from the muscle showed a very rich and apparently pure culture of a bacillus morphologically similar to *B. paludis*, and this bacillus was recovered in cultures.

2.—Approximately 180 c.c. of bacteria-free filtrate of the peritoneal fluid from Sheep W. 42, diluted with an equal volume of saline, were inoculated intravenously into a normal sheep. The animal collapsed

while the material was yet being inoculated and after a few convulsive struggles it died. Death occurred about five minutes after the inoculation had been made. This sheep had been inoculated within a few minutes of the inoculation of the previous animal, and again the carcase was left lying in the field and the post-mortem examination made the following morning, approximately 16 hours after death. The subcutaneous tissues contained a small quantity of gelatinous material. The muscles were moist, but neither soft nor spongy, nor riddled with gas. The difference in the appearance of the subcutaneous, intermuscular, and muscular tissues of this sheep and the corresponding tissues of the sheep inoculated with the unfiltered peritoneal fluid which contained *B. paludis* was most striking. In the present case there was no evidence of changes simulating the appearance of black-quarter or gas gangrene. In other respects, the post-mortem examination resembled that of the previous sheep.

Smears were made from the muscle tissues and these showed a few very large bacteria but they did not resemble *B. paludis*, and in cultures made from the muscles there was no evidence of the presence of that organism.

There is no doubt that the changes found in the muscular, intermuscular, and subcutaneous tissues of the animal which was inoculated with material containing bacteria were produced by the post-mortem generation of those bacteria.

It is only in the " struck " sheep which has been dead some considerable time that changes simulating black-quarter are found. These, being post-mortem changes, dismiss the suggestion that " struck " and black-quarter are similar diseases.

The Experimental Production of " Struck."

The occasional inability to demonstrate pathogenic bacteria in body fluids and tissues of sheep which have died from " struck," and the improbability that the ante-mortem invasion of the body with pathogenic bacteria was responsible for the lesions encountered at or soon after death, led to the experimental inoculation of healthy sheep with the body fluids and tissues of " struck " sheep collected at the time of death.

1.—Portions of three congested mesenteric lymph glands from Sheep W. 26 (tissues sterile), together equivalent to the size of a walnut, were ground up in a mortar with sterile saline, and the emulsion obtained was inoculated intravenously into a sheep. The animal remained well.

2.—Six grams of spleen from Sheep W. 26 were ground up in a mortar with sand and 40 c.c. of saline. Twenty c.c. of this blood-stained suspension of the spleen tissue was inoculated intravenously into a sheep. The animal remained well.

3.—A congested mesenteric lymph gland from Sheep W. 58 (tissues sterile) was ground up in a mortar with 20 c.c. of saline, and the whole was inoculated intravenously into a sheep. The animal remained well.

4.—A congested mesenteric lymph gland from Sheep W. 58 was ground up in a mortar with 20 c.c. of saline, and the whole inoculated subcutaneously

into a sheep. At the seat of inoculation a local reaction occurred, but otherwise the animal remained normal.

5.—The peritoneal fluid from Sheep 26 contained no demonstrable toxin when 6 c.c. were inoculated intravenously into rabbits. Twenty c.c. of the peritoneal fluid from Sheep W. 26 was inoculated into a sheep. The animal remained well.

6.—Ten c.c. of the peritoneal fluid from Sheep W. 57 (*B. paludis* generalised in body) were centrifuged and inoculated intravenously into a rabbit, but no evidence of toxin was obtained.

Two hundred c.c. of the peritoneal fluid from Sheep W. 57, after filtration through a Seitz filter, were inoculated subcutaneously into a sheep. The animal remained well.

7.—Two hundred c.c. of the unfiltered peritoneal fluid from Sheep W. 57, shown by cultural methods to contain *B. paludis*, were inoculated intravenously into a normal sheep. The animal remained well.

8.—Ten c.c. of the peritoneal fluid from Sheep W. 58 were inoculated intravenously into a rabbit, and no toxin was demonstrated.

One hundred and twenty c.c. of the peritoneal fluid from the same sheep were inoculated intraperitoneally into a sheep. The animal remained well.

Summary of the Experimental Inoculation of Normal Sheep with Material Obtained from Sheep Affected with "Struck."

The intravenous and subcutaneous inoculation of sheep with saline emulsions of mesenteric lymph glands and the intravenous inoculation of sheep with emulsion of spleen tissue which have contained no demonstrable bacteria and were derived from sheep which had died or had been killed while suffering from "struck," did not result in the transmission of the disease or in the production of any signs of illness.

The peritoneal fluid from affected sheep used in these transmission experiments was tested for toxin by inoculating moderate quantities of the fluid into rabbits by the intravenous route. No toxin was demonstrated, and the intravenous inoculation of large quantities of the peritoneal fluid into sheep, even though it contained *B. paludis*, as was the case of that derived from Sheep W. 57, was without harmful effect. Further, the intraperitoneal inoculation of peritoneal fluid and the subcutaneous inoculation of filtered peritoneal fluid produced no effect on the experimental animals. The failure to produce the disease in any of the above experiments makes it most unlikely that the disease is an inoculable one caused by a virus present in the affected lymph glands, the spleen, or the characteristic exudates in the peritoneal cavity.

The Oral Administration to Normal Sheep of Body Fluid and Tissue and the Contents of the Alimentary Tract of Animals which had died from "Struck."

1.—The intestinal contents from Sheep W. 26 were collected, and approximately 900 c.c. of these were fed to a sheep. The animal remained well.

2.—Twelve feet of the small intestine, containing ulcerated areas, were chopped into small pieces one-half to two centimeters in length, and fed to a sheep. The animal remained well.

3.—Some of the peritoneal fluid of Sheep W. 42 which contained large numbers of *B. paludis* was mixed with an equal volume of normal saline solution and filtered through a Seitz filter. One c.c. of the filtrate was shown to contain sufficient *B. paludis* toxin to kill a rabbit in a few minutes.

Six hundred c.c. of this peritoneal fluid, unfiltered, and containing *B. paludis*, were fed to a sheep. Fifteen hours later the animal was found dead. Rigor mortis was present, and blood-stained froth and liquid were present at and around the nostrils. The mucous membrane of the conjunctiva was congested. Small areas of gelatinous infiltration were found under the skin of the neck. The muscles were moist, but showed no other abnormalities. The peritoneal cavity contained over one litre of turbid, blood-tinged fluid, and all the organs in this cavity were in a moderately advanced state of putrefaction. The kidneys and liver were soft and foamy, and the spleen was slightly swollen and soft. An excessive quantity of fluid was present in the thoracic cavity. The lungs were soft and decomposed, the apical lobe of each lung being chiefly affected. Doubt was entertained as to whether some of the peritoneal fluid had entered the lungs when the animal was fed and had accelerated the sheep's death, and after this the stomach tube was used in feeding experiments. However, the excess of fluid in the peritoneal cavity encouraged the suspicion that the cause of the animal's death was similar to that which brought about such extensive exudations into the peritoneal cavity of sheep dying from " struck " in the field.

Smears made from the peritoneal fluid of this experimental sheep showed a rich mixture of bacilli, but smears made from the spleen pulp showed large numbers of bacilli morphologically similar to *B. paludis* and a few longer and more slender bacilli ; smears made from the muscle tissue showed an apparently pure culture of bacilli morphologically similar to *B. paludis*.

Cultures made from the spleen and the muscle in both instances gave bacilli and colonies identical with *B. paludis* and its colonies, and the inoculation of a guinea-pig with cultures produced typical *B. paludis* lesions (McEwen, 1930).

Six hundred c.c. of the peritoneal fluid from the experimental sheep were fed to a sheep. The animal remained well.

The contents of the abomasum and the small intestine of the experimental sheep were fed to a sheep. The animal remained well.

4.—The peritoneal fluid of Sheep W. 57 and W. 58 were both devoid of demonstrable *B. paludis* toxin, although cultural tests showed that *B. paludis* was present in the peritoneal fluid of Sheep W. 57. The peritoneal fluids from these two animals were mixed in equal proportions, and 2,500 c.c. of the mixture were given by the mouth to a sheep. The animal remained well.

5.—The contents of the abomasum and the small intestine of Sheep W. 58, amounting in all to 1,500 c.c. of material, were similarly given to a sheep. The animal remained well.

Summary of the Experimental Feeding of Normal Sheep with the Body Fluids and Tissues and the Contents of the Alimentary Tract of Animals which had died from " Struck."

Experiment No. 3 gave encouraging results in spite of the fact that its value may be somewhat discounted by the possibility that some of the material which was given entered the lungs though no conclusive evidence of this was obtainable. From this experiment it would seem that if peritoneal fluid which contains large numbers

of *B. paludis* and its toxin is administered to a healthy sheep, it may bring about death in a matter of a few hours, producing exudations of fluid into the peritoneal cavity not dissimilar to those met with in natural cases.

The oral administration to normal sheep of large quantities of peritoneal fluid derived from an experimental sheep and containing large numbers of bacteria, but not large numbers of *B. paludis*, failed to produce any symptoms, and the administration of a very large quantity of peritoneal fluid from natural cases when the fluid contained no demonstrable toxin and only a very few *B. paludis*, failed to produce any symptoms in a healthy sheep.

The disease was not produced by feeding intestinal and abomasal contents or by feeding the ulcerated small intestine to sheep.

The evidence obtained from the experiments, therefore, would indicate the possibility of producing the disease experimentally by feeding material rich in *B. paludis* and its toxin.

The Oral Administration of Toxic Filtrates of B. paludis *to Sheep.*

The filtrates used in the experiments were obtained from 18 to 24-hour growths in glucose liver broth. All the feeding experiments were conducted with the stomach tube.

1.—Four litres of glucose liver broth exactly comparable to that used for the growth of cultures, but with the *p*H adjusted to 4·6, were given to a sheep. The animal was in no wise upset, and remained healthy.

2.—The filtrate from a culture of *B. paludis* was inoculated intravenously into a rabbit in an amount of 0·5 c.c. This killed the rabbit in six minutes.

One thousand eight hundred c.c. of the filtrate were then given by the stomach tube to a sheep. The animal in all respects remained well.

3.—A culture of *B. paludis* was filtered. The filtrate in 1 c.c. amount was inoculated intravenously into a rabbit. This killed the animal in twenty minutes, and 2 c.c. inoculated intravenously killed a rabbit in eight minutes.

Four thousand five hundred c.c. of this toxic filtrate were given by the stomach tube to a sheep. The animal in all respects remained normal and well.

4.—0·5 c.c. of a *B. paludis* filtrate, when inoculated intravenously into each of two rabbits, produced in ten minutes symptoms of profound collapse and weakness, and death two hours after the inoculation.

Five thousand seven hundred c.c. of this filtrate were given by the stomach tube to a sheep. The animal was not allowed to feed until three hours after the experiment. It was then turned into a field, and immediately it commenced to graze. At the end of the fifth hour it was killed for the purpose of searching the alimentary tract for evidence of toxin. No lesions were found.

The ingesta in the different portions of the alimentary canal were collected and measured. The rumen and reticulum contained 10,000 c.c., the abomasum 350 c.c., the first part of the small intestine 260 c.c., the middle part 700 c.c., and the last part of the small intestine 800 c.c.; the large intestine 1,300 c.c. of material.

The total amount of material in the alimentary tract was approximately 13,400 c.c., and a considerable portion of this must have come from the 5,700 c.c. of filtrate fed to the animal.

From the more than usually liquid character and the large volume of fluid in the rumen and reticulum it appeared as though most of the filtrate

which had been fed was still present in these chambers. The ingesta in the abomasum and first two-thirds of the small intestine was also liquid in character, that in the terminal third of the small intestine was less so, while the contents of the large intestine were moist but not liquid in consistency.

If ingestion, absorption, and defæcation during the five hours preceding death are disregarded, it may be assumed that of the 13,400 c.c. collected at death approximately 7,700 were present in the alimentary tract before feeding the filtrate, and, assuming that the filtrate had been equally mixed throughout the alimentary tract, then every 1·17 c.c. of ingesta should contain an amount of toxin equivalent to that in 0·5 c.c. of the original filtrate, but with the evidence pointing to richer admixture of the filtrate in the rumen and reticulum it is reasonable to suppose that there was a higher concentration of filtrate per cubic centimeter in their contents.

The contents of the different portions of the alimentary tract were centrifuged, and the supernatant fluids inoculated into rabbits with a view to ascertaining whether toxin was present in a concentration which might reasonably be expected, had destruction or alteration of the toxin not already occurred.

Ten c.c. of fluid collected from the contents of the rumen and reticulum were inoculated intravenously into a rabbit. The animal showed no symptoms, and the following day it was well.

Ten c.c. of fluid from the contents of the first portion of the small intestine were inoculated intravenously into a rabbit. Three minutes after the inoculation the animal showed symptoms of weakness and dyspnœa, but in half an hour these had passed off, and the animal was well on the following day.

Ten c.c. of fluid from the middle portion of the small intestine were inoculated intravenously into a rabbit, with a result identical to that of the preceding case.

Even after prolonged centrifuging it was impossible to obtain more than a few cubic centimeters of a turbid fluid from the cream-like contents of the terminal portion of the small intestine. Five c.c. of this fluid inoculated intravenously into a rabbit killed the animal in four minutes. Only six more cubic centimeters of this fluid were available. Three of these were mixed with 2 c.c. of *B. paludis* antiserum, and 3 c.c. were mixed with 2 c.c. of *B. welchii* antiserum. These mixtures, after standing at room temperature for one hour, were inoculated intravenously respectively into each of two rabbits. In each instance the rabbit showed symptoms of weakness a few minutes after the inoculation, but recovered within forty minutes It was therefore concluded that the toxic element in the contents of the small intestine was not *B. paludis* toxin, that none of this toxin was demonstrable, and that in the course of five hours it had been largely, if not completely, destroyed or altered in the alimentary tract.

From these experiments it is assumed that the administration of large quantities of nutrient broth and of filtrates of *B. paludis* containing toxin was harmless for sheep and that the toxin was rapidly altered or destroyed in the alimentary canal, and further, that destruction of toxin occurred in the rumen and reticulum, which makes it improbable that anything but a small amount of unaltered toxin reaches the abomasum when the toxin has been fed.

The Oral Administration of Cultures of B. paludis.

The cultures used were in all instances twenty to twenty-four hour cultures in liver broth, and had a *p*H of 4·5 to 4·6.

The amounts of culture which were given were very large and the numbers of bacteria greatly in excess of what could be ingested naturally by animals, but in any feeding experiment it must be remembered that the great mass of ingesta passes directly into the rumen and reticulum, in which receptacles it becomes diluted with the semi-liquid food there present and only gradually and slowly does it find its way to the true digestive and absorptive portions of the alimentary tract. Further, the cultures consisted entirely of vegetative bacteria and it is possible that many of these are destroyed before reaching the abomasum and small intestine, and, judging from the appearance of smears made from the different portions of the alimentary tract of animals which have been given cultures, this would appear to be the case.

Up to the present, it has not been found possible to obtain cultures of sporulating bacilli suitable for feeding experiments.

In the experiments recorded below large amounts of culture were fed in the hope that if excessive qualities of fluid reached the rumen and reticulum some, at least, would rapidly find its way into the œsophageal groove and reach the abomasum.

1.—The indication that " struck " might be produced by feeding peritoneal fluid which contained large numbers of *B. paludis* led to an attempt to produce the disease by feeding non-toxic peritoneal fluid mixed with a broth culture of the bacterium.

A sheep was fed at hourly intervals with this mixture until six doses had been given, equivalent to 190 c.c. of culture and 120 c.c. of peritoneal fluid. A final dose of 250 c.c. of culture alone was then given. The animal remained well.

2.—One litre of a broth culture was fed to a sheep. The animal remained well.

3.—Two litres of broth culture were fed to a sheep. The animal ceased to feed, diarrhœa ensued, and loss of condition was rapid ; the animal became very weak, and at the end of a week it was not expected to live. After ten days, however, an improvement set in, and during the next week the appetite became normal, and there was a decided improvement in condition. The animal was slaughtered sixteen days after the date of the experimental feeding. The carcase was in a poor condition. The only discoverable lesion was a collection of a yellowish-brown gelatinous material between the left peritoneal surface of the rumen and the abdominal wall. The gelatinous material was traversed by fibrinous strands, some of which were adherent to the rumen and the abdominal wall, while others had free ends extending several inches beyond the gelatinous mass. The remainder of the post-mortem examination was of an entirely negative character.

4.—Four litres of culture were fed to a sheep at 3.30 p.m. The same evening the animal was observed to be lying down more than normally. It was neither grazing nor ruminating, but apart from these signs it was apparently bright and active. The following morning the animal was dull, and appetite and rumination were still in abeyance. By mid-day no further

change was noticeable, and the animal when driven appeared like a healthy sheep. By 7 p.m., a distinct change for the worse was evident, and the animal was standing with its head hanging down and ears drooping, and some time during the night, approximately 36 hours after it had received the dose of culture, it died. When it was found at 8 a.m. in the morning rigor mortis was present and the cornea was opaque. The peripheral parts of the body were cold, but the inguinal regions were still warm to the touch. The peritoneal cavity contained 1,500 c.c. of a yellow and slightly turbid fluid which coagulated on exposure to the air. The peritoneal vessels were intensely injected, and small hæmorrhages were present under the peritoneum. The mucosa of the abomasum in the region of the pylorus was congested, and here and there small hæmorrhagic areas were present in it. The interior of the first 25 ft. of the small intestine showed a number of small areas of congestion, and all of these were deeply injected and suggestive of a stage preceding that of ulceration. The following 15 ft. of the small intestine were acutely congested throughout and the contents were liquid and blood-stained. The remainder of the small intestine showed areas of congestion particularly well defined at the Peyer's patch. The extremity of the cæcum was contracted and the mucous membrane thrown into ridges, and here and there the mucous membrane of this organ appeared to be congested. The colon was contracted, and areas of its mucous membrane were moderately congested. A small quantity of fluid was present in the thoracic cavity. Over the heart were scattered a few subepicardial punctiform hæmorrhages. Under the endocardium of the left ventricle there were numerous hæmorrhagic areas.

Smears made from the peritoneal fluid showed a number of bacilli of *B. paludis* type, five to ten bacteria per field. Smears made from the heart blood, the intestinal lymphatic glands, and the spleen showed no micro-organisms.

Media inoculated with spleen pulp failed to show any sign of bacterial growth, and no ærobic bacteria were recovered from the heart blood and an intestinal lymph gland, but from the heart blood and peritoneal fluid bacilli of *B. paludis* type were recovered in the cultures incubated anærobically.

This case in all its features was identical with a field case of " struck."

5.—Six litres of *B. paludis* culture were given by stomach tube to a sheep at 10 o'clock in the morning. By the afternoon the animal appeared to be a little dull and was not observed to feed. By the evening it showed symptoms of uneasiness, particularly a tendency to stand with its hind legs stretched out in a strained position.

On the following morning, at 7 a.m., the sheep was found dead. The surface of the body was quite cold, the cornea was opaque, and rigor mortis appeared to be passing off. From these indications it was probable that the sheep died in the early hours of the morning, 14 to 16 hours after the administration of the culture. The post-mortem examination revealed a typical case of " struck."

Smears made from the abdominal fluid showed pure cultures of bacteria morphologically similar to *B. paludis*.

The peritoneal fluid, heart blood, and spleen pulp were cultivated, and in each case a bacillus of *B. paludis* type was the only micro-organism recovered. The peritoneal fluid strain was identified as *B. paludis*.

6.—A sheep which had been fed in the stall upon a ration of clover hay and three-quarters of a lb. of linseed cake per day was fed with two litres of

broth culture and one litre of nutrient broth. The animal remained well. It was removed to a small paddock where the grazing was very scanty and a small supplementary ration of oats was given. Two weeks later it was fed with 4½ litres of broth culture. Twenty-four hours after it had been fed the animal was found in a collapsed condition and chloroformed. The post-mortem examination revealed the characteristic picture of " struck."

Smears made from the deposit obtained by centrifuging 50 c.c. of citrated peritoneal fluid showed no bacteria, but masses of leucocytes and an occasional epithelial cell. Smears made from the material in the different portions of the alimentary tract showed bacilli of *B. paludis* type in the abomasum and small intestine only. In the small intestine these bacilli were very numerous.

The heart blood and peritoneal fluid were cultivated. No growth was obtained from the former, but a bacillus morphologically similar to *B. paludis* was obtained from the peritoneal fluid.

Summary of and Discussion on the Experimental Feeding of Sheep with Cultures of B. paludis.

Two sheep, grazing in pasture, were fed by stomach tube with large quantities of culture, four to six litres respectively. A few hours after feeding signs of illness were seen. The animal fed with four litres died within 36 hours. In the case of the animal fed with six litres the illness was more acute and death followed in from 14 to 16 hours.

The post-mortem examinations made on these two sheep revealed lesions which were characteristic of the disease " struck," and the bacteriological examinations of the body fluids and tissues corresponded with those made from natural cases of the disease a few hours after death. In these two experimental sheep ulceration of the small intestine was not present, but this is frequently absent in field cases of " struck."

One sheep fed in the stall was not affected by feeding two litres of culture and one litre of broth, but later this sheep when fed with 4½ litres of culture became affected with typical " struck."

One sheep fed with two litres of culture was rendered seriously ill but eventually recovered.

It is, therefore, considered that the disease has been experimentally produced by administering culture of *B. paludis* by the mouth, but again, as exemplified by the case of the experimental sheep which was killed 24 hours after the administration of culture, the nature and extent of the lesions could not be attributed to the very scanty invasion of the body by bacteria.

In view of the findings obtained, it would appear that the disease might be attributed to the production of toxin by *B. paludis* in the absorptive portions of the alimentary tract, the toxin causing necrosis of the mucous membrane of the intestine and passing into the body giving rise to toxæmia.

The fact that the administration of toxin by the mouth did not produce disease may be attributed to a destruction of the toxin, either before it reaches the abomasum and small intestine, or to a rapid destruction and adsorption in the lumen of the abomasum and small intestine of any toxin which escaped from the rumen and reticulum.

Were the disease to be produced by feeding bacteria without their toxin it would constitute further proof of toxin production in the alimentary canal from whence the toxin reached the body.

The Oral Administration of B. paludis *collected by Centrifuging Broth Cultures, the Bacteria being re-suspended in Fresh Nutrient Broth.*

Fresh nutrient broth was used in the preparation of bacterial suspensions in preference to normal saline.

The animals used had previously been given cultures of *B. paludis* by the mouth whilst they were living in a stable and subsisting on a ration of maize, mangels, and hay. These animals had, contrary to what had been expected, remained well. For the two months prior to the present experiment they had been grazing on permanent pasture.

Two sheep were both fed with bacteria collected by centrifuging 6 litres of broth culture of *B. paludis*. The bacteria were suspended in 5 litres of liver broth and 2 litres of meat infusion broth ; both broths contained 0·4 per cent. glucose.

One sheep, No. 18, was fed with $2\frac{1}{3}$ litres, and the other sheep, No. 19, with $4\frac{2}{3}$ litres. Twelve hours after the material had been fed, the sheep appeared to be well. No. 18, however, was chloroformed, and a post-mortem examination was made. No macroscopic changes were found in any of the organs.

Histological examination of different portions of the small intestine showed in each instance a necrosis of the lining epithelium and a destruction of the epithelium of the more superficial intestinal glands, together with an extensive leucocytic infiltration of the mucous membrane. This infiltration was most extensive in the superficial third of the mucous coat. Eosinophiles were numerous among the infiltrating leucocytes. There were no bacteria in the tissues.

The destruction of the epithelium and the extensive leucocytic infiltration in the mucosa are quite in keeping with the histological picture obtained in cases from the field ; in the latter the process had extended further, and necrosis was more advanced.

The sheep fed with the bacteria suspended in $4\frac{2}{3}$ litres of broth was obviously very ill and unable to move 36 hours after it had been fed. It was, therefore, chloroformed, and a post-mortem examination made ; this was in all respects typical of the disease. In the jejunum there were dark red areas up to 1 cm. in diameter, which suggested the incipient stage of ulceration.

Sections made from the areas of incipient ulceration in the small intestine showed a complete destruction of the mucous membrane down to the submucous coat, the mucous membrane being replaced by an amorphous mass of material detached from the submucosa. Throughout this material and invading the submucous tissues there were considerable numbers of Gram-positive bacilli morphologically similar to *B. paludis*.

In sections made from portions of the intestine which macroscopically appeared normal there was a destruction and necrosis of the superficial third of the mucous coat, together with an extensive leucocytic infiltration throughout the remainder of the mucous membrane, but there was no evidence of any bacterial invasion of the tissues.

Smears made from the peritoneal fluid showed no bacteria, and media inoculated with peritoneal fluid remained sterile.

3.—Three and a half litres of broth culture were fed to a sheep.

4.—The bacteria collected by centrifuging 3½ litres of broth culture were suspended in the same quantity of nutrient broth and fed to a sheep.

Sixteen hours after feeding the animals were ill and disinclined to move, and when made to do so moved in a tucked-up and painful manner, the movements of the limbs being short and stiff. Signs of illness being unmistakable both sheep were chloroformed and post-mortem examinations made.

At the post-mortem the sheep fed with the broth culture presented the characteristic changes associated with " struck." No micro-organisms were found in smears made from the peritoneal fluid, but a bacillus of the *B. paludis* was obtained in cultures therefrom. No other bacteria were present.

Smears made from the contents of the different portions of the alimentary tract indicated that the only part where bacilli morphologically similar to *B. paludis* were present in any number was the abomasum.

The post-mortem examination of the animal fed with the bacteria suspended in nutrient broth showed typical but not advanced lesions. Smears made from the peritoneal fluid showed no micro-organisms, but in cultures a bacillus of *B. paludis* type was obtained. No other bacteria were present.

Smears made from the food material from different portions of the alimentary tract showed numbers of bacilli morphologically resembling *B. paludis* in the deuodenal contents only.

These experiments show that the disease may be produced by feeding bacteria without toxin.

Again, it is not considered probable that in those cases the enteritis, peritonitis, and effusions of fluid in the serous cavities could be attributed to bacterial multiplication in the body.

The only definite foci of bacterial multiplication which have been found in natural and experimental cases have been confined to well defined localised areas of extensive destruction and ulceration of the mucous membrane of the intestine. If bacteria multiplying in the body tissues were the cause of the disease it would be reasonable to assume that the more extensive the ulceration the more acute would be the disease. But this has not been the case in experimental animals, indeed the two which showed the longest survival period were the only ones in which deep localised areas of necrosis of the mucous membrane were found, and in natural cases ulceration has been more common in cases where there was reason to believe that the disease ran a less rapid course. Indirect evidence would, therefore, rule out the bacteria associated with the ulcers as the primary cause of the disease.

B. paludis *Toxin in the Contents of the Alimentary Canal in Field Cases of " Struck."*

Should it be possible to demonstrate *B. paludis* toxin in the contents of the alimentary canal very important evidence would be obtained in support of the theory that the disease is due to the passage of toxin from the alimentary canal into the body.

The rapidity with which a sufficiently large dose of *B. paludis* toxin causes death in the intravenously inoculated rabbit was particularly helpful in these experiments because it enabled the presence, absence, or neutralisation of *B. paludis* toxin in the material tested to be decided

TABLE SHOWING THE RESULTS OF THE SEARCH FOR *B. paludis* TOXIN IN THE PERITONEAL FLUID AND THE CONTENTS OF THE ABOMASUM AND SMALL INTESTINE IN NATURAL CASES OF "STRUCK." INOCULATIONS WERE INTRAVENOUS, AND RABBITS WERE USED.

Sheep from which material was obtained.	*Interval between death and the collection of the material.*	*Material from.*	*Amt. inoculated.*		*Serum*			*Result of inoculation*	*Remarks.*
			Supernatant fluid.	*Filtrate*	*Normal Rabbit.*	B. paludis.	B. welchii.		
W. 49	40 minutes		5 c.c.						*B. paludis* toxin was found in the contents of the small intestine and in considerable concentrations; 5 c.c. of the material containing the filtrate from 2·5 c.c. of intestinal contents killed rabbits in a few minutes.
,,	,,	Abomasum diluted with an equal volume of saline		5 c.c.				None	
,,	,,	Small intestine diluted with an equal volume of saline		6 c.c.				Dead in 6 minutes; typical *B. paludis* intoxication	
,,	,,	,,		6 c.c.	2 c.c.			Dead in 10 minutes	
,,	,,	,,		6 c.c.		2 c.c.		None	
,,	,,	,,		6 c.c.			2 c.c.	Dead in 7 minutes	
,,	,,	,,							
,,	,,	(Another lot of filtrate)		5 c.c.	2 c.c.			Dead in 7 minutes	
,,	,,	,,		5 c.c.		2 c.c.		None	
W. 53	15 minutes	Abomasum		5 c.c.				None	
,,	,,			10 c.c.				None	No evidence of *B. paludis* toxin
,,	,,	First half of small intestine		5 c.c.				None	
,,	,,	Latter half of small intestine		5 c.c.				None	

W. 54	A few minutes	Abomasum	5 c.c.					Dead in 2 minutes	
,,	,,	,,	5 c.c.		2 c.c.			Dead in 2½ minutes	
,,	,,	,,	5 c.c.			2 c.c.		None	*B. paludis* toxin was found in the contents of the abomasum, but it was not demonstrated in the comparatively small quantities of material from the small intestine which were tested
,,	,,	,,	3 c.c.					Weakness followed inoculation, died during the night	
,,	,,	First third of small intestine diluted with third of its volume of saline		3 c.c.				None	
,,	,,	,,	3 c.c.					None	
,,	,,	Middle third of small intestine		5 c.c.				None	
,,	,,	,,		4·5 c.c.				None	
W. 57	A few minutes								A toxin was present in the contents of the small intestine but in low concentration, and toxin neutralisation tests were not made
,,	,,	Abomasum		5 c.c.				None	
,,	,,	,,		10 c.c.				None	
,,	,,	Small intestine plus an equal volume of saline		4 c.c.				One minute after inoculation weakness, improvement followed; apparently normal in 14 minutes. Died overnight	
,,	,,	,,		8 c.c.				Collapse after inoculation. Dead in 2 hours	
,,	,,	,,		10 c.c.				Collapse after inoculation, no improvement, chloroformed at the end of 3 hours	
W. 58	A few mins.								No evidence of *B. paludis* toxin
,,	,,	Small intestine	5 c.c.					None	
,,	,,	,,	10 c.c.					No immediate symptoms but animal died overnight	

within a short time of making the test and so permitted the testing of liquids which were not sterile. The contents of the alimentary canal were, even when diluted with saline, frequently of such a nature that they defied the methods of filtration at one's command. When filtration was impracticable the material was centrifuged and the supernatant fluid without further treatment tested for toxin by inoculating it intravenously into rabbits.

Material from the alimentary tract of five sheep, all of which were typical cases of " struck," has been examined for *B. paludis* toxin. In one case, the toxin was demonstrated in the contents of the abomasum and in another in the contents of the small intestine.

In a third case, Sheep No. 57, a toxin was present in the filtered contents of the small intestine, but its identity was not established.

Unless the toxin was present in a concentration sufficient to kill a rabbit within a few minutes of intravenous inoculation, attempts to establish the specificity of the toxin were not made. It is now thought that too rigorous a standard was set, especially when filtered material was being dealt with, as small doses of *B. paludis* toxin when injected intravenously into rabbits may not kill the animals until 24 to 48 hours have elapsed.

The effects produced upon rabbits by the inoculation of the material from Sheep 57 were in entire agreement with what would have been expected had small quantities of *B. paludis* toxin been inoculated. It is therefore probable that *B. paludis* toxin was present in the contents of the small intestine of Sheep 57.

In the two remaining cases, there was no evidence of *B. paludis* toxin being present.

Search has also been made for *B. paludis* toxin in the contents of the alimentary canal in two experimental animals. One had been infected by feeding 3½ litres of broth culture and the other was infected by feeding the bacteria from 3½ litres of culture suspended in nutrient broth, but in neither of these cases was *B. paludis* toxin demonstrated.

B. paludis *Toxin in the Peritoneal Fluid of " Struck " Sheep.*

The marked injection of and hæmorrhages from the peritoneal vessels, and the turbid effusions of fluid in the peritoneal cavity, the turbidity being due to leucocytes, suggested that these were produced by a localised inflammatory reaction independent of or supplementary to any toxæmia.

Peritoneal fluid was examined for *B. paludis* toxin by inoculating amounts varying from 5 to 12 c.c. intravenously into rabbits.

The toxicity of the peritoneal fluid of Sheep W. 42 has already been noted, the toxin being present in high concentration. But this animal had been dead for six hours, and a very rich culture of the bacteria was found in the fluid. There is little doubt that the toxin was attributable to the bacteria present in the fluid.

Sheep W. 53 presented an interesting case of the absence of toxin from the peritoneal fluid even though bacteria were present in smears

made from the fluid, and no toxin was demonstrated in the peritoneal fluid of two sheep, W. 49 and W. 57, although *B. paludis* was cultivated from the fluid. It would, therefore, appear that small or even moderate numbers of bacteria do not give rise to demonstrable amounts of toxin.

Toxin was demonstrated in 10 c.c. amounts of the peritoneal fluid from Sheep W. 54. The fluid was collected within a few minutes of death and no bacteria could be found in smears. The toxin was neutralised by *B. paludis* antiserum, but not by *B. welchii* antiserum. To find *B. paludis* toxin in the body at the time of death is alone strong corroborative evidence that the toxin is the cause of death, but to find toxin in the peritoneal fluid under conditions where it is not reasonable to attribute its presence to the very scanty number of bacteria in that fluid is remarkable.

Toxin, but in much higher concentration, was found in the abomasal contents of Sheep W. 54.

The presence of toxin in the peritoneal cavity at some stage of the disease would offer an explanation or the peritonitis which is such a constant feature of the disease. The absence of toxin at any one arbitary moment cannot be regarded as an indication that toxin has not been present ; dilution, absorption, and adsorption may have occurred and toxin be no longer demonstrable.

Toxin has been demonstrated in the peritoneal fluid of two experimental sheep, the one fed with bacteria suspended in $3\frac{1}{2}$ litres of nutrient broth and the other fed with $4\frac{1}{2}$ litres of culture. In neither of these sheep were bacteria present in the peritoneal fluid in sufficient numbers to be found in smears. In one case 50 c.c. of citrated peritoneal fluid was centrifuged and the deposit examined, but no bacteria were found. This case illustrates the impossibility of attributing the toxin to bacterial growth in the peritoneal cavity.

If toxin did not originate in the peritoneal cavity it may have been derived from the blood stream or direct from the intestine. Had the toxin been derived from the blood it would be expected in the transudates in both the peritoneal cavity and the thoracic cavity, but this was not the case. When the distinctive and very constant inflammatory reaction of the peritoneum as opposed to the absence of a comparable reaction in the other great serous cavities is considered, it makes it difficult to attribute the peritoneal lesions to anything but a direct passage of toxin from the intestine to the peritoneal cavity—a passage which would be facilitated by the extensive injury inflicted upon the intestine.

Bacteria may be found in the peritoneal fluid while the heart blood appears to be sterile. May not toxin precede the passage of bacteria from the intestine to the peritoneum ?

Toxin may at the same time be absorbed from the intestines by the blood and lymph stream, but the absence of lesions in the liver and the frequent normal appearance of the mesenteric lymph glands would indicate that this does not occur to a marked degree.

General Summary and Conclusions.

The present paper deals with a specific disease of the sheep characterised by an acute and fatal enteritis, peritonitis, and toxæmia. The disease is seasonal, occurring mainly during the late winter and spring months.

The lesions encountered are as follows : Inflammation of the small intestine and occasionally of the abomasum. Ulceration of the small intestine is frequently found, and the cæcum and large colon are sometimes in a state of contraction, their mucous membrane being thrown into folds obliterating the lumen of the bowel. Extensive transudations of fluid are found in the peritoneal cavity, together with injection of and hæmorrhages from the peritoneal vessels. Degenerative changes are occasionally evident in the the kidneys, and transudations of fluid may be present in the thoracic cavity and in the pericardium.

The tissues and body fluids may be sterile, but frequently *B. paludis* may be cultivated from some of them.

As the interval between death and the post-mortem examination increases so, concurrently with post-mortem changes, do the numbers of *B. paludis* increase. Varieties of micro-organisms appear in the peritoneal fluid, but pure and very rich cultures of *B. paludis* are generally present in the muscular tissues even when the post-mortem changes are far advanced. A characteristic and almost specific post-mortem change is met with in the subcutaneous, intermuscular, and muscular tissues. This simulates in a remarkable manner the characteristic ante-mortem changes found in the affected areas in black-quarter of the bovine species. These post-mortem changes in sheep have given rise to the erroneous idea that the disease is a true black-quarter.

The lesions found at the time of death, with the exception of the ulceration in the intestine, have not been attributable to a bacterial invasion of the body tissues. The primary lesion is a necrosis of the superficial mucous membrane in the intestine, but this is not caused by bacteria invading the tissues. The disease is not an inoculable one in the ordinary sense of the term. The disease has been produced by feeding large quantities of broth cultures of *B. paludis* and by feeding the bacteria suspended in fresh nutrient broth.

In natural cases of the disease *B. Paludis* toxin has been demonstrated in the contents of the alimentary tract, and in natural and experimental cases evidence of the passage of this toxin into the body is available, the toxin having been found in the peritoneal fluid under conditions which preclude the production of that toxin in the peritoneal cavity, which suggests that it was derived directly from the contents of the alimentary canal.

The enteritis and peritonitis and toxæmia are ascribable to *B. paludis* toxin derived from the alimentary canal.

Prior to death there is frequently a moderate bacterial invasion

of the body, but this is not the cause of the lesions or the primary cause of the peritonitis or of the toxæmia.

Bacteria may play a secondary part in the ulcerative process in the small intestine.

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"Struck." Enteritis and Peritonitis of Sheep Caused by a Bacterial Toxin Derived from the Alimentary Canal. Paper 2.

BY

A. D. McEWEN

SOUTH EASTERN AGRICULTURAL COLLEGE,
WYE, KENT.

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"STRUCK." ENTERITIS AND PERITONITIS OF SHEEP CAUSED BY A BACTERIAL TOXIN DERIVED FROM THE ALIMENTARY CANAL. PAPER 2.

By A. D. McEwen,

South-Eastern Agricultural College, Wye, Kent.

From investigations conducted on the Romney Marsh, in the spring of 1930, the impression formed was that the number of sheep dying from what the layman calls " struck " was very considerable, and investigations showed that in 75 per cent. of the cases examined the sheep had died from a specific disease, for which the name " struck " was retained. It was attributed to a *B. paludis* toxæmia, the toxin being derived from the alimentary canal and generally, but not necessarily, associated with an invasion of the body tissues by *B. paludis*. (McEwen and Roberts, 1931.)

In 1931 and 1932 it was hoped that field investigations would extend the knowledge of this disease, and during the spring intimate contact was maintained with a number of farms on the Romney Marsh, carrying between 4,000 and 5,000 head of sheep, approximately 2,000 of which had been inoculated at the end of each winter with *B. paludis* anatoxin. It is believed that the majority of the deaths among these flocks regarded by the shepherds as cases of " struck " were reported and post-mortem examinations made. Nevertheless, only 63 suspected cases of " struck " were examined and in only eight was there evidence of the disease associated with *B. paludis*, and from seven of these cases *B. paludis* was isolated.

In both 1931 and 1932 the investigations showed that during that part of the year regarded as the " struck " season the mortality from all diseases considered by the layman as cases of " struck " was not high and that the actual losses from disease associated with *B. paludis* toxæmia or infection was small. There is no doubt that in the 1930 season the losses were very much greater than in either 1931 or 1932, and it is probable that when excessive mortality occurs it is due to the disease associated with a *B. paludis* toxæmia or infection. The evidence accumulated does not cause suspicion of the existence of any other infectious disease.

The Experimental Production of Struck.

In 1930 the disease was produced in sheep by feeding large quantities of broth cultures of *B. paludis*, three litres or more. These large quantities were objectionable, and later further experiments were undertaken in an endeavour to reproduce the

disease by feeding smaller quantities of culture. Feeding experiments were also made with laboratory animals. The disease has been produced in sheep by feeding comparatively small quantities of the bacteria, but attempts to produce disease in guinea-pigs and rabbits by feeding relatively large quantities of the bacteria gave negative results, and attempts to increase the susceptibility of the rabbit to an alimentary infection by variations in diet, *e.g.*, by feeding rabbits upon a diet of lettuce, oats, tapioca, watery extract of liver and peptone solutions, met with no success.

Sheep Feeding Experiments.

(1) An experiment was commenced to test the effect of the repeated administration of small quantities of the bacteria by dosing a number of sheep at short intervals, every second or third day, with small quantities of broth culture of *B. paludis*. This was to be carried on for several weeks, or until some of the sheep died from " struck."

Sixteen sheep at pasture were each given 50 c.c. of broth culture. The animals were dosed in the standing position with the head held horizontally. The culture was administered with great care from a small narrow-necked bottle. No coughing occurred at or soon after the administration of the culture. The following day five of these sheep were found dead and another in a moribund condition was slaughtered. Post-mortem examinations revealed most extensive lesions in the thoracic cavities of all six sheep. The pleural cavity was filled with transudate and gelatinous material, the latter being adherent in places to the lungs, pericardium and parietal pleura. The lungs were œdematous and frothy and areas of these tissues were dark red in colour. Smears made from the cut surface of the lungs showed very large numbers of *B. paludis*. In one sheep the peritoneal cavity contained 500 c.c. of fluid, and the mucosa of the ileum was hyperæmic. The remainder of the cadavers showed no lesions except those in the thoracic cavity.

The six sheep had all suffered from a gas gangrene of the lungs caused through the inhalation of some of the *B. paludis* culture.

The eleven other sheep remained well, but the experiment was not continued.

In the experiments that followed the sheep were fed with quantities of the bacteria collected from the deposits obtained by centrifuging broth cultures of *B. paludis*.

(2) Twelve sheep on pasture were each fed a gelatin capsule of between 1 and 2 c.c. capacity containing the equivalent of the bacteria present in 250 c.c. of broth culture. On the following day three or these sheep were found dead and three more were ill, and by the fourth day eight of the sheep had died. This result had not been anticipated in view of the past difficulty in

setting up alimentary infection except when several litres of culture were fed, and it was not possible to conduct post-mortem examinations until the fourth day, when, despite cold weather, putrefactive changes were advanced in most of the cadavers. One cadaver was, however, in a fresh state and presented the lesions considered typical for a sheep dying from the disease in the field, *viz.*, a copious transudation in the peritoneal cavity, marked injection of the peritoneal vessels, a congested appearance of the small intestine, and moderately congested and œdematous mesenteric lymphatic glands. Cultures from the spleen of this animal remained sterile, and smears from the mesenteric lymphatic glands and other tissues showed no micro-organisms.

Another case where decomposition changes were not advanced presented similar lesions, except that the mesenteric lymph glands were normal in appearance. Smears made from these glands and from the spleen showed bacteria morphologically similar to *B. paludis*.

The remainder of the cadavers were very decomposed, and only four were examined in detail. The lesions in the internal organs were masked by putrefactive changes. The musculature of two of the four cases presented the appearance brought about by the post-mortem multiplication of *B. paludis* and considered indicative of infection with " struck," namely a soft consistence, a dull red colour, and a partly autolysed appearance. Smears from these muscles showed very great numbers of bacteria morphologically identical with *B. paludis*. In the remaining two cases the subcutaneous and muscular tissues presented appearances compatible with the usual putrefactive process only, and smears from the muscles showed no bacteria similar in morphology to *B. paludis*.

This experiment demonstrated that disease could be preduced by administering bacteria collected and contained in a gelatin capsule when the number was at least ten times less than that which had previously been found necessary to produce disease when the bacteria were fed in broth culture.

(3) In a further experiment twelve sheep were each fed with the bacteria collected by centrifuging and obtaining the deposit from 100 c.c. of broth culture of *B. paludis*. The pasty mass was placed on the tongue with a spatula. All of the animals remained well, and this method of feeding was not carried further.

(4) The effect of administering smaller quantities of bacteria contained in gelatin capsules was next tested on five sheep. Each animal was given a capsule containing the bacteria collected from 100 c.c. of broth culture. All of the sheep remained well.

(5) A further experiment was designed to test the effect of repeated small quantities of bacteria administered by the mouth and contained in gelatin capsules. Twenty-two sheep on pastures

were used. Seven of these sheep had been inoculated two months previously each with 10 c.c. of *B. paludis* anatoxin. These animals and seven unvaccinated sheep were each fed with a capsule containing the bacilli equivalent to those present in 20 c.c. of broth culture. The remainder of the 22 animals, eight in number, were left as controls.

The day following the administration of the first capsule (second day) one of the non-vaccinated fed animals was not grazing and on the morning of the third day it died. The post-mortem examination showed a typical case of "struck," and bacilli morphologically similar to *B. paludis* and in apparently pure culture were found in smears from the peritoneal fluid, mesenteric lymph glands, liver and spleen tissue.

On the third day the remainder of the fed animals were again given a gelatin capsule containing the same quantity of bacteria. On the morning of the fifth day a fed unvaccinated animal was found dead. This animal had not been noticed to be ill on the previous day. The post-mortem examination showed a typical case of "struck" except that no microscopic lesions were found in the alimentary canal. Smears made from the kidney and spleen tissues showed no bacilli and in smears from liver tissue a few large putrefactive bacilli were seen, but smears from the mesenteric lymph glands showed numerous bacilli morphologically similar to *B. paludis*. The experimental feedings were not repeated as it was considered that the disease had been produced in two cases. The sheep were kept under observation for some weeks during which time they remained well.

All the above experiments were conducted on sheep grazing at pasture during the late winter and early spring, and in the following experiment the sheep were stall fed. Stall feeding commenced one week before the experimental feeding of culture began.

6. (*a*) Two sheep were fed on a ration of mangles and hay *ad lib.*

(*b*) Two sheep were fed on a ration of 2 lb. of maize and tapioca, equal parts, and a very small quantity of hay.

(*c*) Two sheep were each fed 2 lbs. of crushed oats and a very small ration of hay.

(*d*) Two sheep were each fed 2 lbs. of ground nut and cake and a very small ration of hay.

(*e*) Two sheep subsisting on pasture were also included.

Each of the above animals was fed a capsule containing the bacteria equivalent to those contained in 20 c.c. of broth culture. Three days later the animals were again fed with a capsule containing bacteria. Two days after the administration of the second dose of culture one of the sheep fed on oats and hay and one sheep fed on ground nut and cake and hay were found dead and

in both instances the post-mortem examination showed the lesions associated with " struck " and bacteriological examination demonstrated *B. paludis* in the tissues of these sheep.

The remaining sheep were each fed another capsule but remained well and the experiment was not continued. The experiment, however, demonstrated that stall fed sheep without access to pasture and without green succulent food were susceptible to disease produced by the oral administration of the bacilli.

The complex composition of the pastures and of diets suitable for adult sheep militated against the results of experiments with a limited number of animals giving a reliable index of any increased susceptibility brought about by different kinds of diet, but it was hoped that if a decided or marked difference in the nature of the diet and the resulting products of digestion influenced the growth of *B. paludis* in the alimentary canal and the susceptibility to disease, these might be indicated by feeding lambs upon widely different types of diets.

Preliminary feeding experiments had shown that lambs remained healthy when fed over a period of several weeks on rations of equal parts cows' milk and 17 per cent. lactose solution plus mineral mixture, or equal parts of cow's milk and 17 per cent. solution of casein plus mineral mixture. Furthermore, the animals could be maintained for several days in apparent health when the milk was replaced by either lactose or casein solution, rations of pure carbohydrate and pure protein respectively being fed. However, after remaining on these diets for six days the animals subsisting on pure carbohydrate commenced to suffer from diarrhœa and to become sickly.

Three one week old lambs were fed as follows: Lamb 1 received a ration of cow's milk; Lamb 2 received equal quantities of cow's milk and a 17 per cent. solution of lactose, and Lamb 3 received equal parts of cow's milk and a 17 per cent. solution of casein. The lambs were fed five times a day, and after they had been on the ration for one week 5 c.c. of broth culture of *B. paludis* was added to the ration at each meal for a period of six days. The animals remained healthy, and the milk in the dietaries of Nos. 2 and 3 was then replaced by lactose and casein solutions respectively and the amount of culture fed per meal increased to 20 c.c. The culture was fed for two days and then discontinued. The animals remained on these special diets for another two days. Thereafter they were placed on a normal diet. Throughout the experiment the lambs remained well.

Failure to infect adult sheep with anything but very large quantities of culture fed by the stomach tube may be due to the material entering the rumen, but in lambs the food material passes direct to the abomasum and it was surprising that the experimental lambs should have shown no signs of illness from the ingestion of relatively large quantities of culture. The failure

to infect the lambs is, however, in keeping with the epizootiological evidence, the disease, as far as is known, not occurring in lambs on the Romney Marsh.

Immunisation Experiments.

Preliminary immunisation experiments were conducted with guinea-pigs and rabbits, the animals being inoculated with anatoxin.

The anatoxin was prepared from liver broth cultures of *B. paludis* grown under a vaseline seal. The liver broth was adjusted to a *p*H of appromimately 7·6. The cultures were incubated overnight, in the morning they were cleared by centrifuging and the supernatant fluid filtered through Seitz filters. The filtrate was tested for toxicity and 0·04 c.c. inoculated intravenously into rabbits killed the animals in 15 minutes. The toxic filtrate was tested for sterility by inoculating a quantity into minced meat medium. Sufficient formalin was added to the remainder of the filtrate to give a concentration of 0·5 per cent. and this filtrate incubated at 37° C. for a period of ten days. The toxicity of the filtrate was then tested and if 5 c.c. inoculated intravenously into the rabbit produced no symptoms or illness the detoxicated filtrate or anatoxin was considered ready for use.

A number of guinea-pigs received two inoculations at 14 day intervals of 2 c.c. of anatoxin, and after an appropriate interval these animals and control guinea-pigs were inoculated with small graduated quantities of *B. paludis* broth culture. Both groups behaved with considerable irregularity, some resisting infection, while others succumbed, and as it appeared that accurate information would not be obtained from these methods guinea-pig experiments were not carried further.

Six normal rabbits were bled and their sera tested for antitoxin by inoculating mice intravenously with the mixture of toxin and serum. By the methods employed no antitoxin was detected in the sera of these animals. Three of the rabbits then received a subcutaneous inoculation of a small amount of anatoxin, and three received a larger inoculation. Nineteen days later the animals received a second inoculation of anatoxin. At intervals after both the first and second inoculations the sera of these animals were tested for antitoxin, and finally the resistance of the rabbits to the intravenous inoculation of the toxin was tested.

The toxin used in these antitoxin titration and immunity tests was the dried material obtained from the ammonium sulphate precipitate of filtrate of broth culture of *B. paludis*.

Details of this experiment are given in Table I and show that, as the result of the inoculation of anatoxin, antitoxin appeared in the sera of all the animals, but, on the whole, the rabbits receiving the larger dose of anatoxin showed the greater amount of antitoxin in their sera.

TABLE I.

Rabbit No.	*Serum Tested before Inoculation of Anatoxin.* *Standard Toxin.*				*Amount of Anatoxin. 1st. Inoc.*	*Serum Tested Seventeen Days after First Inoculation of Anatoxin.* *Standard Toxin.*			
	Dilution.	*Amount.*	*Serum.*	*Mice Inoculated iv.*		*Dilution.*	*Amount.*	*Serum*	*Mice Inoculated iv.*
1	1 : 100	0·2 c.c.	0·2 c.c.	+, +, 3-4 hrs.	1 c.c.	1 : 100	0·2 c.c.	0·2 c.c.	+, 3-4 hrs. L.
	1 : 50	„	„	+, +, 5 mins.		1 : 10	0·1 c.c.	„	+, 15 mins.
						1 : 10	0·2 c.c.	„	+, 5 mins.
2	1 : 100	„	„	+, +, 1 hr. 15 mins.	1 c.c.	1 : 100	0·2 c.c.	„	L.L.
	1 : 50	„	„	+, +, 15 mins.		1 : 10	0·1 c.c.	„	+, 2 hrs.
						1 : 10	0·2 c.c.	„	+, 5 mins.
3	1 : 100	„	„	+, +, 1 hr. 15 mins.	1 c.c.	1 : 100	„	„	L.L.
	1 : 50	„	„	+, +, 5 mins.		1 : 10	0·1 c.c.	„	+, 25 mins.
						1 : 10	0·2 c.c.	„	+, 3 mins.
4	1 : 100	„	„	+, 1 hr. 10 mins.		1 : 100	„	„	L.L.
	1 : 100	„	„	+, 5 mins.	10 c.c.	1 : 10	0·1 c.c.	„	L.
	1 : 50	„	„	+, +, 5 mins.		1 : 10	0·2 c.c.	„	L.
						Not diluted	0·1 c.c.	„	+, 5 mins.
5	1 : 50	„	„	+, 2 mins.	10 c.c.	1 : 100	0·2 c.c.	„	L.L.
	1 : 50	„	„	+, 5 mins.		1 : 10	0·1 c.c.	„	L.
						1 : 10	0·2 c.c.	„	L.
						Not diluted	0·1 c.c.	„	+, 1 hr. 30 mins.
6	1 : 100	„	„	+, 20 mins.		1 : 10	„	„	L.
	1 : 100	„	„	+, 4-5 hrs.	10 c.c.	1 : 10	0·2 c.c.	„	L.
	1 : 50	„	„	+, 2 mins.		Not diluted	0·1 c.c.	„	L.L.
	1 : 50	„	„	+, 5 mins.		„	0·2 c.c.	„	L.

TABLE I (*continued*).

Rabbit No.	*Amount of Anatoxin 2nd. Inoc. 19 Days after 1st.*	*Serum Tested 20 Days after Second Inoculation of Anatoxin. Standard Toxin.*				*Intravenous Inoculation of Rabbits with Toxin. 0·4 c.c. of Toxin lethal for normal Rabbits in 15 minutes.*		
		Dilution.	*Amount.*	*Serum.*	*Mice innoc. iv.*	*Time.*	*Amount Toxin.*	*Symptoms, Etc.*
1	2 c.c.	1 : 10	0·2 c.c.	0·2 c.c.	L.	0 mins.	0·4 c.c.	Nil.
		Not diluted	0·1 c.c.	„	+, 15 mins.	5 mins.	„	„
						10 mins.	0·8 c.c.	„
						10 mins.	1·6 c.c.	Slight symptoms recovered.
2	2 c.c.	1 : 10	0·2 c.c.	„	L.	0 mins.	„	Slight symptoms, recovery in 15 mins.
		Not diluted	0·1 c.c.	„	+, 2 mins.	20 mins.	„	Symptoms in 10 mins. + in half-an-hour.
3	2 c.c.	1 : 10	0·2 c.c.	„	+, 1 hr.	0 mins.	0·8 c.c.	Symptoms in 8 mins. + in 20 mins.
4	10 c.c.	Not diluted	0·1 c.c.	„	+, 4 hrs.	0 mins.	1·6 c.c.	Nil.
			0·2 c.c.	„	+, 5 mins.	15 mins.	„	Symptoms in 5 mins. + in 10 mins.
5	10 c.c.	Not diluted	0·1 c.c.	„	L.	0 mins.	3·2 c.c.	Nil.
		„	0·2 c.c.	„	+ following morning	15 mins.	„	„
6	10 c.c.	„	0·1 c.c.	„	L.	0 mins.	6·4 c.c.	Nil.
		„	0·2 c.c.	„	L.			
		„	0·4 c.c.	„	L.			
		Double strength.	0·4 c.c.	„	+, 5 mins.			

Serum and toxin mixtures were allowed to stand 1 hour at room temperature before these were inoculated into the mice.

As the degree of immunity produced by the inoculation of the anatoxin appeared to bear some relationship to the quantity of the anatoxin inoculated, it was of interest to ascertain whether concentrated dried toxin dissolved in comparatively small quantities of saline, filtered and treated with formalin, would possess a definite antigenic value. If a definite value were demonstrated it would be possible to immunise animals by the inoculation of a comparatively small volume of the material.

Dried toxin was diluted in saline and filtered through a Berkefeld candle. The filtrate was ten times as toxic as the filtrates of broth culture used in the preparation of the ordinary anatoxin. The concentrated filtrate of toxin was detoxicated by formalin in the usual manner. Three rabbits with no demonstrable antitoxin in their sera were each inoculated subcutaneously with 1 c.c.

Fourteen days later their sera were tested for antitoxin. None was demonstrated in the sera of two of the animals but antitoxin was present in the serum of the third rabbit. The animals received a second inoculation of 1 c.c. and 18 days later their sera were tested.

TABLE II.

RABBITS INOCULATED WITH ANATOXIN PREPARED FROM CONCENTRATED TOXIN. TWO INOCULATIONS OF 1 C.C. WERE GIVEN.

Rabbit.	*Serum Tested* 14 *Days after First Inoculation.* *Standard Toxin.*			
	Dilution.	*Amount.*	*Serum.*	*Mice.*
10	1 : 50 1 : 10	0·2 c.c. „	0·2 c.c. „	+, +, in 5 hrs. +, 5 mins. +, 11 mins.
11	1 : 50 1 : 10	0·2 c.c. „	0·2 c.c. „	+, +, few hours. +, +, 2 mins.
12	1 : 50 1 : 10	0·2 c.c. „	0·2 c.c. „	L.L. L.L.

Rabbit.	*Serum Tested* 18 *Days after Second Inoculation.* *Standard Toxin.*			
	Dilution.	*Amount.*	*Serum.*	*Mice.*
10	1 : 10 Not diluted	0·2 c.c. „	0·2 c.c. „	L.L. +, +, 3 mins.
11	1 : 10	0·2 c.c.	0·2 c.c.	+, 5 mins. +, few hours.
12	Not diluted „	0·2 c.c. „	0·2 c.c. „	L. +, few hours.

The results of these tests are shown in Table II, and it will be seen that in two of the rabbits sera very appreciable amounts of antitoxin were present.

Immunisation of Sheep.

In the following experiments all the sheep were bled before they were inoculated or used as control animals and their sera was tested for antitoxin by methods similar to those employed in testing rabbit sera. In no instance was detectable antitoxin found in the sera of sheep before immunisation.

For the purpose of description the unit of toxin is regarded as the amount of toxin required to kill mice within 15 minutes of intravenous inoculation. The volume of toxin inoculated was maintained at 0·2 c.c., concentration only being the variable factor. The volume of serum inoculated was also kept constant namely 0·2 c.c. The antitoxin units mentioned below refer to the number of units per c.c. of serum and where 0·2 c.c. of serum completely neutralised a quantity of toxin lethal for mice within 15 minutes of intravenous inoculation the serum is assumed to contain at least five units of antitoxin. Similarly, had it completely neutralised five lethal doses of toxin the antitoxin content of the serum would be placed at 25 units. When complete neutralisation of toxin did not occur but the test animals survived for some hours this is taken as indicating the presence of some antitoxin but insufficient to be expressed in terms of units.

The anatoxin used for inoculation of sheep 1 to 4 inclusive was the same as that used for the immunisation of guinea-pigs and rabbits No. 1 to 6 inclusive. The anatoxin used in the remainder of the sheep immunisation experiments, except where otherwise stated, was prepared in a comparable manner to that previously described but the toxicity of the broth culture filtrates was tested on mice and 0·004 c.c. was lethal for these animals within 15 minutes of intravenous inoculation. After the addition of formalin and incubation at 37° C., 0·5 c.c. of the anatoxin was harmless for mice on intravenous inoculation.

Experiment 1.—Two sheep, Nos. 1 and 2, were each inoculated subcutaneously with 1 c.c. of anatoxin. Eighteen days later the serum of these animals was tested for antitoxin but none was demonstrable. On the same day they received a second inoculation of anatoxin, and on the seventh and again on the seventeenth days after the second inoculation their sera were tested, but in neither instance was any antitoxin found. The experiment was not carried further with these animals.

The sheep Nos. 2 and 4 were inoculated at the same intervals as sheep Nos. 1 and 2, but with 10 c.c. of anatoxin. Following upon the first inoculation no appreciable quantity of antitoxin was found in their sera, but after the second inoculation antitoxin was present in the serum of sheep No. 4, 1 c.c. of serum containing 25 units of antitoxin. The

serum of sheep No. 3, however, contained but little antitoxin, insufficient to be expressed in terms of units.

These two sheep received a further inoculation of anatoxin three weeks after the second inoculation had been given, but in neither instance did the third inoculation materially affect the antitoxin content of their sera. Four months after the final inoculation the immunity of sheep Nos. 3 and 4 was tested by feeding the sheep each with 3½ litres of broth culture of *B. paludis*. A control sheep, No. 5, was fed with the same amount of culture. The control sheep was found dead 20 hours later and the post-mortem examination revealed the typical picture of "struck." Twenty-six hours after feeding, sheep No. 3 was acutely ill and unable to stand. It was therefore chloroformed and a post-mortem examination made, and the characteristic lesions of " struck " were found. Sheep No. 4, showing antitoxin in its serum, remained well.

Experiment 2.—The effect of inoculating sheep with larger quantities of anatoxin was then tested, and the sheep Nos. 6 and 7 each received two inoculations of 50 c.c. of anatoxin at an interval of 15 days. Before the second inoculation was given the sheep were bled and their sera tested. The sera from sheep Nos. 6 and 7 both contained at least 25 units of antitoxin per c.c. Fifteen days after the second inoculation their sera were again tested and there appeared to be some slight increase in the amount of antitoxin present. The immunity to *B. paludis* culture administered by stomach tube was then tested. Sheep No. 6 and a control sheep, No. 8, were fed each with 3½ litres of broth culture. The following morning the control sheep was dull and apathetic and 24 hours after the administration of the culture it died. The post-mortem examination revealed a typical case of " struck." Sheep No. 6 remained well. Two days after sheep Nos. 6 and 8 had been fed, the vaccinated sheep No. 7 and a control No. 9 were each fed 3½ litres of broth culture of *B. paludis,* comparable in every respect to the culture fed to Nos. 6 and 8. Sheep No. 7 remained well, but the control sheep No. 9 was found dead the morning following the administration of culture. The post-mortem examination of sheep No. 9 did not show the characteristic lesion of " struck." The tissues of the head and face were œdematous and the mucous membrane of the interalveolar space showed surface erosion probably caused by the mouth gag when the culture was administered by stomach tube. Smears made from the œdematous fluid in this neighbourhood showed numerous micrococci. There was no excess of fluid in the peritoneal cavity nor was there injection of the peritoneal vessels. In the small intestine the Peyer's patches appeared congested and one of the mediastinal lymphatic glands was congested and œdematous. No other lesions were found. Pure cultures of *B. paludis* were isolated from a mediastinal lymphatic gland and from the spleen. The death of this sheep cannot be attributed to an infection with *B. paludis*, though it is probable that the bacteria found in the body represented an ante-mortem invasion and that had there been no localised infection of the tissues of the face and head the animal would have succumbed to a *B. paludis* infection of alimentary origin.

The evidence from the experiments, however, indicated that vaccinated animals with anatoxin in their serum withstood the

feeding of culture which was generally lethal for non-vaccinated animals or for animals without antitoxin in their serum.

Experiment 3.—The effect of the inoculation of 10 c.c. anatoxin was again tried.

Two sheep, Nos. 10 and 11, were inoculated each with 10 c.c. and 15 days later they were bled and their sera tested. In neither instance was there an appreciable quantity of antitoxin in the sera. The sheep then received a second inoculation of 10 c.c. anatoxin and 15 days later their sera were obtained and tested. The serum from sheep No. 10 showed no antitoxin but the sample from sheep No. 11 showed at least 25 units per c.c. It was decided to give sheep No. 10 a third inoculation of anatoxin, but by mistake this sheep was inoculated with anaculture. No increase in the amount of antitoxin present in the serum resulted and the animal was dismissed from experiment.

Sheep No. 11 and a control, No. 12, were each fed with 3½ litres of broth culture of *B. paludis*. The vaccinated sheep remained well but the control sheep No. 12 died between 24 and 32 hours after being fed. The post-mortem and bacteriological findings in this case were typical of those for a " struck " sheep examined several hours after death. This experiment further confirmed the opinion that vaccinated animals with antitoxin in their sera withstood the oral administration of culture of *B. paludis* which was lethal for animals whose serum contained no antitoxin.

Experiment 4.—The immunising value of anaculture was next tested. The original toxicity of the culture was the same as that of the filtrate from which anatoxin was prepared. The culture was rich in bacteria and its opacity was the same as that of a suspension of *B. coli* containing 5,000,000,000 bacteria per cubic centimeter. 0·5 per cent. formalin was added to the culture and the culture was incubated 10 days, by which time no toxin was demonstrable on the intravenous inoculation of 0·5 c.c. into mice, and sterility tests showed that the bacteria were no longer viable.

Two sheep, Nos. 13 and 14, were each inoculated at 16-day intervals with 50 c.c. of anaculture. Just before the second inoculation was made the sheep were bled and their sera tested for antitoxin. In both sera 25 units of antitoxin were demonstrated. Twenty-six days after the second inoculation the sera were tested but no appreciable increase in antitoxin was found. Five months after the second inoculation the sera were again tested and in the case of sheep No. 14 there was a decline in the amount of antitoxin, only five units being demonstrated.

The immunity of these sheep was now tested, in the case of sheep No. 13 by feeding it with culture and of sheep No. 14 by the intramuscular inoculation of *B. paludis* culture.

Sheep No. 13 and a control sheep, No. 15, were each fed 3½ litres of broth culture of *B. paludis*. Sheep No. 13 remained well, but sheep No. 15 was found dead 24 hours later, and the post-mortem examination showed the typical " struck " lesions.

Six days after it had received the feed of culture No. 13, along with No. 14 and a control animal, No. 16, were each inoculated intramuscularly in the thigh with 0·5 c.c. of a 1 : 8 saline dilution of broth culture of *B. paludis*. Sheep Nos. 14 and 16 were found dead the following morning. The post-mortem examinations of these two sheep

showed in each case extensive gas gangrene lesions of the inoculated leg caused by *B. paludis*. Sheep No. 13 was lame for several days but it recovered completely. The experiment again demonstrated the resistance of sheep whose serum was relatively rich in antitoxin and indicated that such animals withstood the severe test of the intramuscular inoculation of culture, whereas sheep vaccinated with anaculture but showing little antitoxin in their serum succumbed.

The anaculture did not appear to be superior to anatoxin for the immunisation of sheep.

Experiment 5.—Four sheep were included in a further experiment, both anatoxin and anaculture being used and the amount of the inoculation in each instance reduced to 5 c.c.

Sheep Nos. 17 and 18 were inoculated each with 5 c.c. anatoxin. Eighteen days later 5 units of antitoxin per c.c. of serum were demonstrated for sheep No. 17 and 25 units per c.c. of serum for sheep No. 18.

Sheep Nos. 19 and 20 were each inoculated with 5 c.c. of anaculture. Eighteen days later the sera from these animals was tested and each was shown to contain but little antitoxin—less than five units per c.c.

The immunity of sheep Nos. 18 and 20 was then tested by the intramuscular inoculation of 0·25 c.c. of broth culture of *B. paludis*. A control sheep, No. 21, was also inoculated. The control sheep died in 15 hours. Sheep No. 20 died a few hours later. Sheep No. 18 survived. Post-mortem examinations of Nos. 20 and 21 showed extensive gas gangrene lesions of the inoculated leg due to a *B. paludis* infection.

The immunity of sheep Nos. 17 and 19 was tested by feeding each with three litres of broth culture. A control, No. 22, was also fed with three litres of the culture. Eighteen hours later the control sheep died. Nos. 17 and 19 remained well. The post-mortem examination of sheep No. 22 showed a typical case of " struck."

In this experiment no superiority was shown by anaculture as an immunising agent. Again resistance to infection is seen to be linked to the presence of antitoxin in the serum. The experiment is interesting in that it shows that considerable resistance was produced in at least two of the sheep, Nos. 17 and 18, by the inoculation of only 5 c.c. of anatoxin.

Anatoxin is not considered to possess any antigenic superiority over anaculture, but it is considered preferable for use in field vaccinations on the Romney Marsh because the former is more certain to be sterile than the latter, and it is desirable to use a vaccine of assured sterility so that the vaccine itself may be exonerated from blame should gas gangrene result from its careless use. (Roberts and McEwen, 1932.)

The preparation of large quantities of culture filtrate for the production of anatoxin is tedious because of the preliminary centrifuging required to clear the culture before filtration can be satisfactorily carried out. Were filtered anaculture to prove as efficient an antigen as the ordinary anatoxin this would afford an easier method of preparing vaccine for use in the field, because upon standing the bacteria in the anaculture settle out, leaving a

clear supernatant fluid which may be syphoned off and filtered without preliminary centrifuging.

Filtered anaculture was, therefore, prepared from the same batch of anaculture used in the previous experiments.

The sheep Nos. 23 and 24 were each inoculated subcutaneously with 10 c.c. Eighteen days after inoculation their sera were tested and no antitoxin found. A second inoculation of 10 c.c. was given and 14 days later the sera tested. Sheep No. 23 showed 5 units of antitoxin per c.c. Three months after the second inoculation the sera of these animals were again tested but in neither case was antitoxin detected in a preciable quantity.

Sheep No. 23 was then fed 3½ litres of broth culture and 24 hours later it was found dead. The post-mortem examination showed the characteristic lesions of " struck."

Sheep No. 24 was inoculated intramuscularly with 0·5 c.c. of a 1 :40 saline dilution of broth culture of *B. paludis* and died from an acute gas gangrene infection. At the same time sheep No. 4 which had previously resisted feeding with 3½ litres of *B. paludis* broth culture and a control sheep No. 26 were also inoculated intramuscularly with the same quantity of the culture. Sheep No. 4 remained well, but the control sheep died of acute gas gangrene infection.

From the results of these experiments it was not considered advisable to continue further work with filtered anaculture.

As the inoculation of rabbits with concentrated anatoxin had given encouraging results, the effect of the inoculation of concentrated anatoxin was tested on two sheep. Two sheep, Nos. 27 and 28, each received two inoculations of 1 c.c. of concentrated anatoxin at an interval of 18 days. Their sera was tested 18 days after the first inoculation and again ten days after the second inoculation. In neither case was any antitoxin found in the serum. The experiment was not carried further, and no more work was done with a concentrated type of anatoxin.

These experiments demonstrated the great variations in the response of sheep to the inoculation of both anaculture and anatoxin, some animals developing more antitoxin than others, and again the methods used failed to detect any antitoxin in the sera of yet other sheep. The higher the antitoxin content of the serum the more resistant were the animals to the drastic methods employed to test immunity, namely, administering large quantities— 3 to 3½ litres of broth culture by the mouth and the intramuscular inoculation of culture. In animals showing 25 units of antitoxin or more per c.c. of serum the resistance to infection was complete, and sheep with at least 5 units of antitoxin in their serum per cubic centimetre, withstood attempts to infect them by feeding large quantities of culture.

The degree of resistance to infection runs parallel with the concentration of antitoxin in the serum, and resistance or

immunity appears to be obtained just as effectively by vaccination with anatoxin as with anaculture. Although immunity was produced by the single inoculation of a dose of 5 c.c. of anatoxin, larger doses—50 c.c. may be more effective. Further, a second inoculation generally increases the antitoxin content of the serum. As these experiments were designed with the purpose of gaining

TABLE III.

No. of Experiment.	*No. of Sheep.*	*Units of Antitoxin in Serum before Inoculation.*	*Inoculation.*	*Units of Antitoxin in Serum after Inoculation.*	*Method of Treating Immunity.*	*Result.*
(1)	1	None	Anatoxin, 2 inoculations each 1 c.c.	None		
	2	„	Do. do.	„		
	3	„	Do. do. 10 c.c.	„	Fed 3½ litres broth culture.	Infected and chloroformed.
	4	„	Do. do. 10 c.c.	25	Do.	Remained well.
	5	„	None	None	Do.	Died in 20 hours.
(2)	6	„	Anatoxin, 2 inoculations each 50 c.c.	25	Do.	Remained well.
	8	„	None	None	Do.	Died in 24 hours.
	7	„	Anatoxin, 2 inoculations each 50 c.c.	25	Do.	Remained well.
	9	„	None	None	Do.	Died in 24 hours, but disease not typical.
(3)	10	,	Anatoxin, 2 inoculations each of 10 c.c. Inoculated a third time, but no antitoxin demonstrated and dismissed from experiment.	None	Do.	
	11	„	Do. do. 10 c.c.	25	Do.	Remained well.
	12	„	None	None	Do.	Died 24 to 36 hours later.
(4)	13	„	Anaculture, 2 inoculations each of 50 c.c.	25	Fed 3½ litres of broth culture.	Remained well.
	15	„	None	None	Fed 3½ litres of broth culture.	Dead in 24 hours.
	14	„	Anaculture, 2 inoculations each 50 c.c.	25 5 mths. later only 5.	Inoculated intramuscularly with culture	Dead 15 hours later.
	16	„	None	None	Do.	Do.
	13	„	See above	„	Do. One week after having been fed culture.	Lame but recovered.
(5)	17	„	Anatoxin, 1 inoculation of 5 c.c.	5	Fed 3 litres of broth culture.	Remained well.
	19	„	Anaculture, 1 inoculation of 5 c.c.	Less than 5	Do.	Do.
	22	„	None	None	Do.	Dead 18 hours later.
	18	„	Anatoxin, 1 inoculation of 5 c.c.	5	Inoculated intramuscularly with culture.	Lame but recovered.
	20	„	Anaculture, 1 inoculation of 5 c.c.	Less than 5.	Do.	Dead within 15 hours.
	21	„	None	None	Do.	Do.
(6)	23	„	Filtrate anaculture, 2 inoculations of 10 c.c.	5 3 mths. later none.	Fed 3½ litres of broth culture.	Dead in 24 hours.
	24	„	Do. do.	None	Inoculated intramuscularly	Dead in 24 hours later.
	26	„	None	„	Do.	Do.
	4	„	See Experiment 1	„	Do.	Lame but recovered.
(7)	27	„	Concentrated anatoxin, 2 inoculations of 1 c.c.	„		
	28	„	Do. do.	„		

information which would be of value in planning field vaccinations to protect sheep against a natural attack of the disease, little consideration was given to the effect of more than two inoculations as it would not be practicable to inoculate sheep in the field more than twice; indeed it would be preferable if immunisation could be accomplished by one inoculation. Therefore, in the preparation of anatoxin for use in the field the toxin content of the original culture should be as high as possible, a strain of bacterium capable of potent toxin production and a suitable medium being used.

The experiments indicated that immunised sheep remained protected in some cases for at least five months, but that during this time a decline in the degree of immunity probably occurred.

The experiments held out the hope that the inoculation of sheep in the field with anatoxin will afford a means of protecting the animals against " struck." Even though some experimental animals failed to respond as well as others, and were not sufficiently protected to withstand the drastic feeding or inoculation tests, they may, nevertheless, have been sufficiently protected to withstand a natural field infection.

Field Vaccination Experiments 1931 *and* 1932.

Anatoxin was prepared in the manner already described.

In January and February of 1931, sheep on different areas of the Romney Marsh were vaccinated, some receiving one inoculation, and others receiving two inoculations of 10 c.c. each of anatoxin at an interval of ten to 14 days. In each field where vaccinated sheep were, an equal number of non-vaccinated sheep were kept as controls. Altogether approximately 4,000 sheep were under experiment, 2,000 being vaccinated animals.

It was hoped that at the end of the season the owners and shepherds would be able to supply information regarding the value of the vaccinations, but very few sheep died during the season and, although accurate figures were not kept by the shepherds or owners, it was evident that no material difference in the number of deaths in the vaccinated and control groups occurred.

Field vaccinations were again made in 1932, and in January and February, 2,048 sheep were vaccinated, two inoculations, each of 10 c.c. of anatoxin being given. In each field with the vaccinated sheep an equal number of sheep were left unvaccinated to act as controls.

During the period of the year when " struck " is expected, namely, from the middle of February to the end of May, every sheep dying in these flocks was autopsied and when found necessary a bacteriological examination was made. Excluding one or two deaths directly attributable to lambing, only 20 vaccinated

and 26 control animals died. One of the vaccinated animals presented all the characteristic lesions of "struck." This animal was examined immediately after death and culture media were inoculated with tissue from the liver, the spleen, the mesenteric lymph glands, and heart blood, but these all remained sterile. *B. paludis* was isolated from five of the control sheep and it is considered that these sheep died from "struck." In no other case was there any evidence that death was due to "struck." On account of the comparative rareness of the disease during the past two seasons, 1931 and 1932, the field vaccination experiments yielded no definite information.

General Summary.

During 1930, 75 per cent. of sheep regarded by the shepherds and owners as having died from "struck" were considered to have suffered from a *B. paludis* toxæmia or infection.

During the 1931 and 1932 seasons comparatively few sheep died on the Romney Marsh, and the examination of 63 cases of alleged "struck" showed that only a relatively small percentage were due to a *B. paludis* toxæmia or infection. It is considered likely that in seasons when mortality is high and the owners and shepherds complain of many losses from "struck," the excessive mortality is due to a *B. paludis* toxæmia or infection, but in other seasons the mortality on the Romney Marsh is little, if at all, higher than in other parts of the country where specific contagious disease is not suspected and that in such seasons deaths from *B. paludis* toxæmia or infection are very rare occurrences and of negligible economic importance.

The disease may be produced in sheep by the administering of comparatively small numbers of *B. paludis* when these are enclosed in a small, 1 or 2 c.c. gelatin capsule.

The disease was not produced in sucking lambs by feeding these with quantities of *B. paludis* broth culture along with their food. Wide variations in the composition of the diet did not affect the apparent insusceptibility of lambs to the ingestion of cultures of *B. paludis*.

Rabbits and guinea-pigs fed with relatively large quantities of *B. paludis* remained well.

Guinea-pigs have not been found suitable animals for immunisation experiments when their immunity was tested by the inoculation of cultures of *B. paludis*.

Rabbits proved suitable animals for preliminary experiments, their immunity response being gauged by the estimation of the antitoxin content of their sera and their resistance to the intravenous inoculation of toxin.

Sheep inoculated with anatoxin or anaculture showed considerable variation in their resulting immunity. Anaculture,

sterilised bacteria plus toxoid, was not found to be a superior antigen to anatoxin.

Sheep inoculated with anatoxin or anaculture and showing appreciable quantities of antitoxin in their sera withstood the oral administration of quantities of broth culture of *B. paludis* which were lethal for control sheep, and some withstood the intramuscular inoculation of quantities of *B. paludis* culture, lethal for less highly immunised sheep and for control animals.

Sheep inoculated with anatoxin or anaculture and showing little or no antitoxin in their sera did not withstand the above very severe tests.

The majority of sheep respond to the inoculation of anatoxin or anaculture by producing antitoxin. Therefore, when animals in the field are vaccinated with anatoxin or anaculture the majority may be expected to respond by producing antitoxin and to be immune to natural infection. Furthermore, it is possible that those whose serum contains insufficient antitoxin to be detected by the methods employed in these investigations, may, nevertheless, be sufficiently immune to resist a natural infection.

Field vaccination experiments during 1931 and 1932 have yielded no information because of the comparative absence of disease during these years.

Reference.

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Gas Gangrene Infections of Sheep

BY

R. S. ROBERTS and A. D. McEWEN.

The South-Eastern Agricultural College, Wye, Kent.

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GAS GANGRENE INFECTIONS OF SHEEP.

By R. S. Roberts and A. D. McEwen.

The South-Eastern Agricultural College, Wye, Kent.

In a former paper dealing with a disease of sheep on the Romney Marsh termed " struck," reference was made to a condition popularly termed " gangrene " or " ganger " which may affect the ewe a day or two after lambing or the lamb a day or two after castration or docking, and occasionally appears in a flock of sheep soon after shearing. There is, however, always a clear history of wound infection. (McEwen and Roberts, 1931).

The infectious nature of the disease is recognised by farmers and shepherds, but in so far as the bacteriology of the condition is concerned no investigations appear to have been made until one of us (A.D.M.) in 1928-1929 studied material received from a number of cases and also had the opportunity to make a post-mortem and bacteriological examination upon a ewe a few minutes after death. *B. chauvœi* and *V. septique* were the pathogenic micro-organisms isolated at these examinations. The results of the investigations were in agreement with those of researches upon similar diseases in other countries, notably Germany.

During the springs of 1930-31 further cases were investigated, and in nearly all instances the post-mortem examinations and the collection of the material for bacteriological examination were made personally.

Certain fields on the Romney Marsh are reputed to be particularly dangerous for the parturient ewe, but there is not the same feeling regarding the relationship to the pasture where the disease occurs after docking, castration, or shearing. The disease occurs with greater regularity in the ewe after lambing and is then of greater economic importance. The possibility that it is transmitted by the hands of the shepherd and his lambing equipment is recognised because the majority of ewes succumbing to the disease have received assistance at lambing. The incidence of the disease, therefore, may be affected by factors quite irrespective of the degree of infection of the ground upon which the animals are grazing. One owner, whose observations we respect, states that when the disease assumes alarming proportions it may be stopped by moving all the pregnant animals to fresh ground and not permitting the shepherd to go near them, allowing the ewes to lamb by themselves if the services of a fresh shepherd are not available. It is only where the disease is assuming alarming proportions that such drastic methods would be resorted to. The disease occurs only during the latter half of the lambing season. It has not been possible to obtain figures of the percentage of losses caused by it. On one farm in 1930 thirty ewes were lost out of 300, but this proportion is in great excess of the

mortality over the whole area of the Romney Marsh. It is possible that the losses fluctuate between 2 and 3 per cent. The mortality among other classes of animals from a wound gas gangrene is of less economic importance, though when a considerable number of deaths occur simultaneously they cause alarm.

Gas Gangrene in the Parturient Ewe.

Clinical Features.

Symptoms may become apparent at any time from 6 to 22 hours after lambing, but they are seldom noticed until 24 to 36 hours have elapsed. The animal ceases to feed and ruminate, remains apart from the flock, and neglects its lamb. Breathing becomes accelerated and the vulva and surrounding tissues tumefied and bluish-red in colour. The tumefaction and discolouration spreads and involves the whole perineal region, extending from the under surface of the tail above to the udder below. Drops of blood-tinged serous fluid exude through the skin. The respirations are laboured. After this stage is reached the ewe is no longer capable of standing, coma supervenes, and death quickly follows. The whole clinical course of the disease does not exceed 48 hours and in the majority of cases it is much shorter, death occurring on the third day after lambing.

Post-mortem Appearances.

A characteristic feature of the post-mortem examination is that lesions are chiefly confined to the perineal region. The skin of these parts is discoloured, varying from a pink to a purplish tint. Beads of pink serous fluid may exude through the skin. The wool over the affected area is easily detached and may be found matted in little patches with this serous exudate. Palpation of the affected region reveals underlying œdema and tumefaction but not crepitation, and on incision the subcutaneous and intermuscular tissues are found infiltrated with a large amount of gelatinous liquid, pink to purple in colour. Gas is absent from these infiltrations. The muscular tissues of the affected parts may show slight tumefaction and darkening but these lesions are not constant. There are cases, however, where the musculature is extensively involved and the muscles of the loins and of the thighs swollen and very dark in colour, and in some instances portions of the affected muscle are emphysematous, presenting the typical blackquarter appearance. The sourish smell associated with blackquarter is occasionally noted. The mucosa of the vagina generally shows areas of erosion and necrosis, these occurring most regularly in the neighbourhood of the fornix. The cervix of the uterus may show similar lesions and its folds be œdematous. The uterus in its gross appearance is generally comparable with a healthy organ examined at a similar period after parturition, but its walls and the broad ligament may be markedly œdematous and thickened. Occasionally a moderate excess of peritoneal fluid is encountered. This fluid is sometimes slightly blood-tinged.

When the animal has been dead for some hours the subcutaneous tissues over the whole body or the greater part thereof are puffy from distension with gas and a moderate quantity of semi-fluid material. The skeletal muscles of the body are dark, moist, and friable and the intermuscular septæ contain gas and gelatinous fluid. Changes of a putrefactive nature are advanced in the abdominal and thoracic cavities, both of which contain an excess of blood-tinged fluid, and there is hæmoglobin staining of the viscera, imparting to them a false inflammatory appearance.

Wound Gas Gangrene of Lambs.

Animals generally die on the second or third day following the operation of castration and docking. Clinically they do not show any features which might not be expected from an acute wound infection and a septicæmic condition. Few lesions were encountered in lambs. The subcutaneous tissues were moist and the thigh muscles slightly darker in colour than normal. No lesions were found in any of the internal organs.

We have not had an opportunity to examine a case of gas gangrene following shearing, but from the reports given by shepherds the disease appears to be similar to that encountered in lambs.

Bacteriology.

The bacteriological methods employed during the 1928-29 seasons consisted in isolating pure strains of bacteria from specimens of muscle sent to the laboratory by veterinary surgeons, farmers, and shepherds, and in identifying them.

The identification of the bacteria was based upon the type of colonies produced on serum agar surface slants, in shake liver agar, and shake blood agar; the cultural reaction in broth containing minced meat, nutrient broth, alkaline egg broth, milk media, milk containing a portion of brain tissue, broth containing brain tissue and a piece of iron wire, broth containing a cube of coagulated egg albumen; and fermentation reactions, glucose, lactose, saccharose, salicin, inulin, glycerine, and mannite being used as the test substances.

During the season the only pathogenic anærobes encountered were *B. chauvœi* and *V. septique*. These two micro-organisms were distinguished one from another by the following reactions :—

	B. chauvœi.	*V. septique.*
Surface colonies ...	Discrete, round or slightly irregular borders.	Spreading, with long filamentous processes.
Shake agar	No growth	Large woolly colonies.
Shake blood agar ...	Pinpoint compound colonies.	Large woolly colonies.
Milk	No growth	Good growth, clot and gas.
Milk and brain tissue ...	Large smooth clot, small volume of gas.	Stormy clot, large volume of gas.
Saccharose	Fermented	Not fermented.
Salicin	Not fermented	Fermented.

During the 1930 season the strains of pathogenic anærobes isolated were studied in a similar manner except that the blood agar medium was omitted.

Each strain as it was isolated was inoculated into guinea-pigs, and the characteristic lesion for *B. chauvœi* and *V. septique* infection were reproduced in every instance when these respective micro-organisms were inoculated.

In the 1931 season we did not consider it necessary to undertake detailed cultural examinations with a variety of media in order to establish the identity of the strains, and in the case of *B. chauvœi*, examinations were confined to the character of the growth and its microscopical appearance in minced medium and on serum agar slants. The *V. septique* strains were studied in the same two media, and their fermentation reactions were also ascertained. Again all strains were inoculated into guinea-pigs, the lesions were noted, and smears made from the seat of inoculation and peritoneal surface of the liver were examined. Further, with the *B. chauvœi* strains isolated in 1930 and 1931 identification of the micro-organism was confirmed by the neutralisation of the pathogenic action of the cultures by small quantities of specific immune serum, guinea-pigs being used as the test animals and suitable controls included for every strain tested. The immune serum was prepared by the repeated inoculation of a sheep with large quantities of *B. chauvœi* (ovine strain) anaculture.

The *B. chauvœi* strains when first isolated were all very pathogenic, 0·1 c.c. killing guinea-pigs in 16 to 24 hours. With storage and sub-cultivation there was a considerable loss of virulence. All the *V. septique* strains were highly pathogenic for guinea-pigs, and there has been no suggestion of their having become any less virulent by storage or sub-cultivation.

In only one instance has a pathogenic anærobic bacillus other than *B. chauvœi* and *V. septique* been encountered in these investigations. This was isolated in pure culture from the pale subcutaneous fluid and also from the muscles around the vagina of a ewe which had died three days after lambing. The organism gave the characteristic reaction for *B. œdematiens* in culture. It produced the typical pale œdematous lesions on inoculation into guinea-pigs. Further, its pathogenic action was neutralised by *B. œdematiens* serum. It is regarded as a typical strain of *B. œdematiens*. This case was particularly interesting in that the appearance of the lesions in the ewe was suggestive of a *B. œdematiens* infection. The lesions differed from those encountered on all the other occasions in the greater œdema of the region of the vulva and in the palor of the skin of the affected parts. Again, the infiltrating fluid was a pale straw colour, the subcutaneous vessels were injected, and the musculature was paler than in normal animals.

During the preliminary examinations conducted in 1928-29 non-pathogenic bacteria had been encountered on a number of

occasions, but these were considered as in all probability contaminants and discarded. The results of the last two seasons' examinations appear to justify this assumption, as when the material was fresh the invading micro-organism was present in a pure state.

Details of the history of the case were often omitted by the senders of the material in the 1928-29 investigations, and it is possible that some of these specimens came from sheep which had died from some disease other than gas gangrene. In five instances, however, it was definitely stated that the ewe had died from " gangrene." From three of these *V. septique* was recovered, and from the remaining two *B. chauvœi.* Twenty-eight specimens received without a history showed *V. septique* in 18 instances and *B. chauvœi* in ten. In our researches on the disease " struck " we have never recovered *B. chauvœi* from the carcase of affected animals even after these had been lying on the field several hours, but *V. septique* was found on several occasions in carcases which were examined some hours after death and it was regarded as a post-mortem invader (McEwen and Roberts, 1931). It is therefore improbable that the ten cases from which *B. chauvœi* was isolated were anything but cases of gangrene, though doubt may be entertained regarding the nature of the disease in the 18 instances where *V. septique* was isolated. These results indicated that *B. chauvœi* and *V. septique* were both capable of causing the disease. In 1930-31 *B. chauvœi* was encountered more frequently than *V. septique* and the latter was never found invading the tissues of sheep when these were examined at or very soon after death.

The results of the examinations made upon material collected personally, and where the interval elapsing after death was known, are recorded in the Table.

The table reveals the relationship between the species of bacterium isolated and the time which elapsed between death and the bacteriological examination. Thus, 13 cases examined within 10 hours of death all gave cultures of *B. chauvœi*, this being the only pathogenic bacterium isolated, and in all but one instance it was found in the local lesion in a pure state. In six cases the exact time of death was not known, since the sheep had died in the night and material was collected the following day, but the interval would be about 10-16 hours. In two of these cases *B. chauvœi* was the only micro-organism isolated ; in one instance both *B. chauvœi* and *V. septique* were recovered ; in two cases *V. septique* alone was isolated, and in the sixth case *B. œdematiens* was found to be the invading micro-organism. One case examined at the time of death showed a pure staphylococcal infection.

The preponderance of *B. chauvœi* over *V. septique* is illustrated in these investigations where material was collected personally, and this fact, coupled with the striking absence of *V. septique* in fresh cases, raises the query as to whether *V. septique* was anything more than a post-mortem invader.

TABLE

Case.	*Time between Parturition and Death.*	*History of Lambing.*	*Time between Death and the Examination.*	*Distribution of Lesions.*	*Smear Examinations of Local Lesions.*	*Materials from which Cultures were made.*	*Bacteria Isolated.*
1	3 days	Normal	15 minutes	Vagina, cervix, walls of uterus and broad ligament, subcutis of perineum and tail	B. chauvœi type	Muscle of thigh and subcutaneous tissues of tail, heart blood, and spleen	B. chauvœi
2	2-3 days	Assisted	Immediately	Vulva and perineum	B. chauvœi type	Local lesion, heart blood, and peritoneal fluid	B. chauvœi
3	2-3 days	—	Killed and examined immediately	Perineum, vulva, and muscles of thigh	B. chauvœi type	Subcutaneous tissues of vulva, muscle, and peritoneal fluid	B. chauvœi
4	3 days	—	Immediately	Perineum, vulva, and muscles of thigh showed typical blackquarter tumefaction.	B. chauvœi type	Muscle and heart blood	B. chauvœi
5	24 hours	Normal	Immediately	Vulva and perineum, purulent condition of mammary gland	B. chauvœi type; mammary gland, a short, stout Gram-positive bacillus	Muscle and subcutaneous tissue of vulva	B. chauvœi
6	4 days	—	Killed and examined immediately	Vulva and perineum. Moderate inflammation of the small intestine	Cocci	Local lesion, heart blood, spleen, and peritoneal fluid	Staphylococcus
7	2 days	Normal	1 hour	Perineum, cervix of uterus, muscles of thigh	B. chauvœi type	Muscle and heart blood	B. chauvœi
8	2 days	Assisted	2 hours	Vulva perineum, and muscles of thigh	B. chauvœi type	Subcutaneous tissues of vulva and muscle	B. chauvœi

9	2 days	Assisted	7 hours	P[illegible]eum, vagina, cervix of uterus	B. chauvœi type	Subcutaneous ti[illegible] sues of local lesion and muscles of thigh	B. chauvœi
10	2-3 days	—	7 hours	Vulva, perineum, and tail; œdema of uterus	B. chauvœi type	Subcutaneous tissues of local lesion	B. chauvœi
11	—	—	7 hours	Vulva, perineum, and tail	B. chauvœi type	Subcutaneous tissues of local lesion and thigh muscle	B. chauvœi
12	Exact time not known, probably 3-4 days	—	6-10 hours	Carcase had been skinned and eviscerated by shepherd. When examined the thigh muscles showed extensive lesions	B. chauvœi type	Muscle of thigh	B. chauvœi
13	3 days	Normal	6-10 hours	Perineal region, vulva, lumbar and thigh muscles	B. chauvœi type	Muscle	B. chauvœi
14	2-3 days	Assisted	Found dead in morning	Vulva and perineum	B. chauvœi type	Local lesion	B. chauvœi
15	3 days	—	Found dead in morning	Vulva and perineum	B. chauvœi type	Local lesion	B. chauvœi and V. septique
16	2 days	—	Found dead in morning	Vulva and perineal region. Underlying muscles ve.y pale	An anærobic type of bacillus not similar to B. chauvœi	Subcutaneous tissues of vulva and muscle	B. œdematiens
17	24 hours	Assisted	Found dead in morning	Perineum and vulva	B. chauvœi type	Local lesion	V. septique and another anærobic bacillus not identified
18	3 days	Assisted	Found dead in morning	Vulva, perineum and vagina	Considered of V. septique type	Local lesion	V. septique
19	2-3 days	—	Found dead in morning	Vulva and perineum	B. chauvœi type	Local lesion	B. chauvœi
20	2-3 days	—	24 hours	Vulva and perineum	B. chauvœi type	Local lesion	V. septique

During the 1929 season material from five lambs said to have died from " gangrene " following upon castration was examined, and in each case *B. chauvœi* was the only pathogenic bacterium isolated. In 1930 four similar cases were obtained personally, and in each case *B. chauvœi* was found in the muscle of the hind limbs in abundant and pure culture.

GAS GANGRENE IN SHEEP FOLLOWING UPON VACCINATION.

During the past season, 1931, experimental vaccination of approximately 1,500 sheep was carried out against " struck " with an anatoxin prepared from bacteria-free filtrate of cultures of *B. paludis*. On all but one farm these inoculations had been made personally, the sheep having been held in a standing position and inoculated into the subcutaneous tissues at the back of the shoulders after the skin had been cleaned with methylated spirits containing carbolic acid or with weak tincture of iodine. No ill effects followed these vaccinations, great care having been taken to prevent, as far as possible, contamination of the hands with soil and to keep the vaccine and instruments used in making the inoculations sterile. On one farm, however, vaccinations were not made personally and 120 ewes were inoculated in the thigh, the animals being caught and held in a very muddy pound ; 15 per cent. of these sheep died from gas gangrene as a direct result of the inoculation. Five of these animals were examined and four showed a pure infection with *B. chauvœi*. With the fifth there was a mixed infection, but neither *B. chauvœi*, *V. septique*, nor any recognised species of pathogenic anærobe was isolated. This illustrates very clearly the heavy contamination of the soil on the Romney Marsh and the extreme susceptibility of sheep to infection with *B. chauvœi*, and from our observations it would appear that under natural conditions sheep are more susceptible to *B. chauvœi* than to infection with *V. septique* or any other species of bacterium of the gas gangrene group.

It is surprising that spontaneous cases of blackquarter analogous to the bovine disease are not of frequent occurrence. Cave (1907), in his researches on " struck," states that that disease is in all respects a blackquarter caused by the blackquarter bacillus, but from his description of the bacillus it would appear that he was not dealing with *B. chauvœi* but with a micro-organism " stout and with rounded ends," which more closely resembles *B. paludis*. In the course of examinations upon an extensive number of sheep which have died suddenly or after a short illness, and without a history of wounding, we have recovered *B. chauvœi* on one occasion only. This animal was a two-year-old ewe, and it had been observed to be ill only a few hours before death and had been eviscerated and hung up by the owner at the time of death. It was examined 12 hours after death, and the left thigh showed a typical blackquarter lesion ; no other abnormalities were found.

It is interesting to note that on this farm, though on a different field, blackquarter had appeared in a group of 20 recently purchased

eighteen-months-old heifers and six had succumbed to the disease. We obtained a strain of *B. chauvœi* from one of these heifers which had died a day or two after removal from the Marsh pasture; culturally it was indistinguishable from the ovine strains, and *B. chauvœi* anti-serum produced with an ovine strain protected guinea-pigs against this bovine strain.

Discussion.

When the results of the bacteriological examinations of all the material received are considered independently of the history of the cases, the two micro-organisms which appear to have ætiological significance are *B. chauvœi* and *V. septique*. This is in substantial agreement with the findings of Miessner and Albretcht 1924, Ræbiger and Speigl 1924, Manniger 1924, Knall 1924, and Wolters 1927, all these authors attributing significance to *V. septique* though agreeing that the majority of the cases of " gas œdema " in sheep are caused by *B. chauvœi*.

Miessner and Albrecht examined 56 specimens of muscle sent to the Veterinary High School at Hanover. From 54·28 per cent. *B. chauvœi* were isolated, 18·7 per cent. showed *V. septique*, and 18·7 per cent. *B. chauvœi* and *V. septique*, 8·42 per cent. gave *B. welchii* or *B. welchii* with *B. chauvœi* or *V. septique*. In eight instances no bacteria were isolated. Ræbiger and Speigl report that 48 to 94 per cent. of cases are due to *B. chauvœi* and 6 to 52 per cent. to *V. septique*. Manniger, in a flock where death followed castration, isolated *B. chauvœi* from the cases he examined; on the same farm eleven sheep died after shearing and *B. chauvœi* was isolated and regarded as the causal micro-organism, but in another flock, where the disease followed parturition, *V. septique* was recovered. Knall reports the isolation of *B. chauvœi* from 22 cases, *B. chauvœi* and *V. septique* from two cases, *B. chauvœi*, *V. septique* and another micro-organism from two cases, and in one instance *V. septique*. Wolters studied 44 cases, and in 30 he found a *B. chauvœi* infection, but in thirteen *V. septique* was recovered and in one *B. sporogenes*. The greater importance of *B. chauvœi* is further emphasised by the findings of Marsh 1919 and 1923, and of Marsh, Welsh, and Jungherr 1928. The former author reported two outbreaks of gas gangrene in sheep due to *B. chauvœi*, and Marsh, Welsh, and Jungherr recorded sixteen outbreaks; a bacteriological examination was made upon one sheep from each of thirteen of these outbreaks and *B. chauvœi* was isolated in ten instances.

If it were possible to attribute ætiological significance to any pathogenic anærobe found in the tissues at the time of examination, then the view that *V. septique* is the cause of the disease in a number of instances could be accepted. But when consideration is given only to those cases which we have examined and where the history is known, it is found that in no instance was *V. septique* recovered when the bacteriological examination was made at the time of

death or very soon after death, and it was only after a considerable lapse of time that *V. septique* showed itself.

In our report on our investigations upon "struck" we recorded the isolation of *V. septique* from a number of cases examined some time after death and under circumstances where it could be regarded as a post-mortem invader of the carcase. This conclusion was justifiable because the characteristic lesions of "struck" are produced by the toxin of *B. paludis* and so permit the ruling out of *V. septique* as the cause of the disease in the few instances where it was recovered. The same definite conclusions regarding *V. septique* are not applicable in cases of gas gangrene on account of the close similarity between the lesions produced by *B. chauvœi* and *V. septique* in the subcutaneous and muscular tissues when these micro-organisms are experimentally inoculated into sheep. However, should *V. septique* appear as a post-mortem invader in the disease "struck" it is likely to play a similar part in other diseases of sheep grazing upon the same ground; and should the initial lesion of gas gangrene be due to *B. chauvœi*, but *V. septique* be present in the lesion as a post-mortem invader, it is probable that the latter organism will obscure the presence of the former at bacteriological examination. Microscopically they frequently cannot be distinguished with any degree of certainty, and in culture *V. septique* would rapidly outgrow *B. chauvœi*, making it impossible to detect the presence of that organism. We have tried to separate *B. chauvœi* from *V. septique* by cultural means when they were present in a mixture in varying proportions ranging from one part of *B. chauvœi* and one part of *V. septique* to 1,280 parts of *B. chauvœi* and one of *V. septique*. These mixed cultures when sown on serum agar slants invariably produced a surface growth of *V. septique* which obscured any developing *B. chauvœi* colonies; in minced meat medium the cultures morphologically resembled *V. septique* rather than *B. chauvœi*, and sowing from the minced meat medium cultures to serum agar slants resulted in the production of a spreading growth of *V. septique*. We consider that in many instances a *B. chauvœi* infection would be completely masked were there even a moderate post-mortem invasion of the part with *V. septique*, and in those rare cases when *V. septique* and *B. chauvœi* have both been isolated that the former bacterium was probably present in very small numbers and may have been present in one lot of seed material and not in another. Therefore, *V. septique* cannot definitely be incriminated as the cause of gas gangrene in sheep until it is isolated from perfectly fresh material.

The mere ubiquity of highly pathogenic anærobic bacteria—micro-organisms which on parenteral inoculation of the sheep are as pathogenic as *B. chauvœi*—does not necessarily mean that the micro-organisms will be found to cause natural cases of gas gangrene in these animals. Cases of gas gangrene and "struck" occur on the same pastures and at the same time of the year. The contamination of these pastures by *B. paludis* must in many instances be heavy

and the hands of the shepherd are at times no doubt contaminated by *B. paludis* while he attends his ewes at lambing, yet we have not found gas gangrene infection to be caused by this organism or by any bacteria of the *B. welchii* type. Again, the frequent recovery of *V. septique* indicates that the organism is very widely distributed on the pastures, but there is no conclusive evidence that it causes the disease. A case of particular interest was the one in which a typical strain of *B. œdematiens* was isolated. The case unfortunately was not obtained at the time of death, but from the appearance of the local lesions it is considered that *B. œdematiens* was the causal micro-organism.

Summary.

A bacteriological examination has been made of 68 cases of gas gangrene, a disease which causes serious mortality among sheep on the Romney Marsh.

The disease is a common one in ewes shortly after lambing, the infection gaining entrance to the tissues through abrasions or wounds of the genital organs. *B. chauvœi* is usually the cause.

In gas gangrene in lambs the infection gains entrance to the body through wounds inflicted at castration or docking. Nine cases have been studied and *B. chauvœi* was recovered in all of them.

Gas gangrene has been found following vaccination with sterile material when the vaccination was done under adverse conditions and there was a great risk of contamination from the soil. Five of these cases were examined and *B. chauvœi* was the causal micro-organism in four cases. The fifth case showed a mixed bacterial infection, but no recognised species of bacterium of the gas gangrene group was recovered.

The soil of the Romney Marsh appears to be heavily contaminated by *B. chauvœi*, *V. septique*, and *B. paludis*.

B. paludis or bacilli of the *B. welchii* type have not been encountered in cases of gas gangrene. *B. œdematiens* appeared to be responsible in one case.

V. septique has not been proved to be the cause of gas gangrene, and it has not been definitely incriminated in any of our investigations into sheep diseases. It is frequently recoverable, apparently as a post-mortem invader, from the bodies of animals that have been dead for some time.

Despite the wide distribution of *B. chauvœi* throughout the Romney Marsh, only one case has been encountered in which it is probable that the infection did not occur through a wound but spontaneously in the manner of blackquarter in the bovine.

Prophylactic measures should be directed primarily against a *B. chauvœi* infection.

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GAS GANGRENE INFECTIONS OF SHEEP: PASSIVE IMMUNISATION.

By A. D. McEwen and R. S. Roberts.
South-Eastern Agricultural College, Wye, Kent.

Investigations on the ætiology of gas gangrene of sheep were described in a previous paper* and *B. chauvœi* was found to be the common causal micro-organism against which prophylactic measures should be taken. During the 1932 lambing season further cases have been studied, and the results confirm the observations previously made.

The bacteriology of the cases examined this year is summarised below. The remainder of the paper is concerned with passive immunisation against *B. chauvœi* by methods which appear to be of practicable application in the district where the disease has been encountered, *viz.*, the Romney Marsh.

Summary of Cases in 1932.

Altogether 19 cases have been investigated. Material for bacteriological examination was collected personally and in 18 instances post-mortem examinations were made. The bacteria were recovered either from affected muscle tissue or from the subcutaneous or intramuscular infiltrations.

Eleven cases of post-parturient gas gangrene in the ewe were obtained. *B. chauvœi* was the only pathogenic micro-organism recovered from seven of these, but *B, chauvœi* and *V septique* were isolated in three instances, and in one instance *B. chauvœi* and a bacillus which was probably *B. welchii*. In the latter instance *B. welchii* was not observed in smears made from the original material; these indicated a *B. chauvœi* infection only. A *B. chauvœi* infection was found in a wether sheep which contracted gas gangrene a few days after the fleece had been clipped from around the hind quarters. Seven cases of the disease in lambs following docking or castration and docking, showed in five instances a *B. chauvœi* infection, while from the remaining two both *B. chauvœi* and *V. septique* were isolated.

In the previous paper the significance of the recovery of *V. septique* was discussed, and it was emphasised that, had *V. septique* and *B. chauvœi* both been present in the original material, the former owing to its more vigorous growth and greater pathogenicity for laboratory animals, would mask the presence of the latter when simple cultural means or animal inoculations were used to isolate and identify the organisms. A consideration of the evidence then obtained indicated the probability of *V. septique* being a post-mortem or secondary invader and not the primary cause of the disease.

*Roberts, R. S., and McEwen, A. D. Jl. Comp. Path & Ther., **44**, 180.

When gas gangrene is experimentally produced in sheep by intramuscular inoculation with either *V. septique* or *B. chauvœi* the macroscopic local lesions are very similar, but in the *V. septique* infection there is generally congestion of the mucosa of the abomasum and of parts of the small intestine, while in the *B. chauvœi* infection these are rarely observed. Therefore, in field cases post-mortem examinations may assist as a guide to the differentiation of the causal micro-organism should this be either *V. septique* or *B. chauvœi*. Further, microscopical examinations of smears from local lesions are of differential aid, as *B. chauvœi* appears as a more slender and elegant micro-organism than *V. septique*, and these microscopical differences are generally apparent to an examiner familiar with the appearance of both micro-organisms when growing in muscle tissue. In all the nineteen cases examined during the past season the evidence gathered from post-mortem examinations and the examinations of smears from local lesions indicated a *B. chauvœi* infection, and it is considered that when *V. septique* was isolated it had been present in the body as a secondary invader and not as the causal micro-organism.

The immunisation of cattle against *B. chauvœi* infection is generally successful and, a priori, it would appear that sheep could likewise be immunised against ovine strains of the bacillus, but were it possible to immunise sheep successfully it is doubtful whether it would be extensively practised in the area where we have encountered the disease. In all classes of sheep the disease is most irregular in its appearance, and farmers frankly admit that even if the disease could be controlled by the inoculation of the whole flock they would rather take the risk of a number of animals contracting the disease than incur the labour and expense of inoculation.

Parturient ewes receiving assistance at lambing suffer more from gas gangrene than do other classes of sheep. If it were possible for the shepherd to protect these animals by inoculating them with immune serum either before or immediately after giving assistance a practical method for saving considerable numbers of animals would be available, and experiments have therefore been undertaken with a view to ascertaining if this could be accomplished. Controlled experiments have demonstrated the value of immune serum in preventing the appearance of an inoculation gas gangrene.

General Methods.

Infection with *B. chauvœi* was produced by inoculating the experimental animals with quantities of diffusion shell broth culture. The cultures used were obtained from a virulent strain of the organism recovered from a sheep which had died from gas gangrene in 1930, and the virulence of the strain was maintained by recovering the micro-organism from time to time from the muscle lesions of inoculated control sheep. After incubation the cultures to be used for infective inoculations were kept in the ice chest, where their virulence remained at an approximately constant level for several weeks, during which time they were available as a supply of infective material.

For each experiment as detailed in the protocols all inoculations were made on one and the same date unless the contrary is expressly stated.

When mice were used the culture was inoculated subcutaneously and with guinea-pigs and sheep inoculations were made intramuscularly.

In attempts to estimate the lethal dose for mice the animals were employed in groups each containing three animals, all members of a group being inoculated with the same quantity of culture. The amounts of culture inoculated per mouse per group were 0·01 c.c., 0·05 c.c., 0·1 c.c., 0·3 c.c., and 0·4 c.c. Fatalities occurred in each group except that in which each mouse received 0·01 c.c., but the mortality was not complete in any group and therefore in further experiments with these animals a relatively large dose of culture was inoculated and by using a number of animals an effort was made to obtain data of some value.

In the case of the guinea-pig 0·5 c.c. of culture was generally fatal and this quantity or 1 c.c. was used in immunity experiments.

With the first experiments on sheep an infective dose of culture of 0·5 c.c. was given, but in subsequent experiments the quantity was reduced to 0·25 c.c. or 0·0625 c.c. the smaller amount almost invariably being lethal.

In 1931 a polyvalent horse serum was prepared, a mixture of equal parts of formalinised culture of four strains of *B. chauvœi* isolated from sheep being used as antigen. The strain used for the infective inoculation was not included in this polyvalent antigen. The following year a further supply of serum was required. By this time the strain used for infective inoculations was the only one which had maintained its full virulence and capacity of vigorous growth

TABLE I.

SERUM AND CULTURE MIXED BEFORE INOCULATION.

a.	*No. of Guinea Pigs.*	*Serum*, 1931.	*Culture.*	*Result.*
	2	0·1 c.c.	0·5 c.c.	L.L.
	4	0·05 c.c.	0·5 c.c.	L.L.L.L.
	6	———	0·5 c.c.	D.D.D.D.D.D.
———		*Serum*, 1932.		
b.	3	0·05 c.c.	1 c.c.	L.L.L.
———	3	0·025 c.c.	·1 c.c.	L.L.L.
	3	———	1 c.c.	D.D.D.
	2	0·016 c.c.	1 c.c.	L.L.
	2	0·0125 c.c.	1 c.c.	L.L.
	2	———	1 c.c.	D.D.
	2	0·01 c.c.	1 c.c.	L.L.
	2	0·008 c.c.	1 c.c.	L.L.
	1	———	1 c.c.	Extensive lesions but recovered.

Section *b* of this experiment was carried out in three parts on three consecutive days, the same culture being used each day.

in broth medium, and formalinised cultures of it were inoculated into the previously immunised horse and a further supply of serum drawn in 1932.

Preliminary demonstrations of antibody in the sera were obtained from guinea-pigs inoculated with mixtures of culture and varying quantities of sera which had been allowed to stand at room temperature for one hour. In 1931 the preliminary test was made before completion of the immunising process.

Using methods similar to those employed above, a comparison was made between the serum prepared in 1931 against ovine strains of the bacillus and a commercial serum prepared presumably against a bovine strain of the micro-organism. The results are given in Table II and indicate the superiority of the 1931 serum.

TABLE II.

No. of Guinea Pigs.	*Serum.*	*Amount of Serum.*	*Culture.*	*Result.*
3	1931	0·2 c.c.	1 c.c.	All lived.
3	Commercial	0·2 c.c.	1 c.c.	Two died.
7	1931	0·1 c.c.	1 c.c.	One died.
7	Commercial	0·1 c.c.	1 c.c.	Five died.
6	1931	0·05 c.c.	1 c.c.	Five died.
6	Commercial	0·05 c.c.	1 c.c.	All died.
3	1931	0·025 c.c.	1 c.c.	All died.
3	Commerical	0·025 c.c.	1 c.c.	All died.
2	Normal horse serum	0·2 c.c.	1 c.c.	All died.
2	Do.	0·1 c.c.	1 c.c.	All died.
2	Do.	0·05 c.c.	1 c.c.	All died.
4	———	———	1 c.c.	All died.

A similar experiment was carried out in mice but without showing any decided difference in the neutralising powers of the two sera.

TABLE III.

No. of Mice.	*Serum.*	*Amount of Serum.*	*Culture.*	*Result.*
3	1931	0·05 c.c.	0·5 c.c.	All lived.
3	Commercial	0·05 c.c.	0·5 c.c.	All lived.
3	1931	0·025 c.c.	0·5 c.c.	Two died.
3	Commercial	0·025 c.c.	0·5 c.c.	One died.
3	1931	0·0125 c.c.	0·5 c.c.	All died.
3	Commercial	0·0125 c.c.	0·5 c.c.	All died.

As these and other experiments reported in the following pages indicated that the serum prepared against ovine strains of the bacillus was of greater potency in neutralising the pathogenicity of ovine strains of *B. chauvœi*, the greater portion of the experimental work was carried out with sera prepared against ovine strains of the organism and only these sera were used in the field.

Passive Immunity Experiments on Laboratory Animals. The simultaneous Inoculation of Serum and Culture.

Guinea-pigs and mice were inoculated with culture and at another part of the body with serum. When a sufficiently large dose of serum was administered to guinea-pigs the inoculation disease did not develop. Mice were not protected by a comparatively small amount of serum inoculated subcutaneously, but when the serum was given by the intravenous route an appreciable protection was evidenced.

TABLE IV.

No. of Guinea Pigs.	*Culture.*	*Serum, 1931.*	*Result.*
3	0·5 c.c. intramuscularly	3 c.c. subcutaneously	D.L.L.
3	,, ,,	6 c.c. ,,	L.L.L.
3	,, ,,	None	D.D.D.
No. of Mice.			
6	0·35 c.c. subcutaneously	0·5 c.c. ,,	D.D.D.D.D.D.
6	,, ,,	1 c.c. ,,	D.D.D.D.L.L.
6	,, ,,	None ,,	D.D.D.D.L.L.
6	,, ,,	0·5 c.c. intravenously	D.L.L.L.L.L.
6	,, ,,	None	D.D.D.D.D.D.

With both species of animals the amount of culture inoculated was large, and when the comparative size of the animals is considered it is evident that the amount inoculated into the mice was relatively much larger than the quantity given to guinea-pigs. In view of this it is not surprising that the comparatively small quantity of serum inoculated subcutaneously into mice failed to confer protection. The success attendant upon the intravenous inoculation of serum into mice was, however, encouraging.

The Effect of a Time Interval between the Inoculation of Culture and the Inoculation of Serum.

Guinea-pigs and mice were inoculated with culture, and then and also two hours and four hours later groups of these animals received serum. In the case of mice serum was withheld until the lapse of even longer intervals of time. On the whole the results indicated that the delayed inoculation of serum gave the animals some protection.

From the Table V it will be seen that the guinea-pigs behaved in an irregular manner, though possibly some protection was conferred by serum even when this had been withheld for four hours after the infective inoculation had been made. Mice also gave somewhat irregular results, mortality not being complete among the control animals. The experiment on mice was carried out in two parts on two consecutive days. On the second day the infective dose was increased in amount in the hope that the control animals would succumb, and on the same day in attempting to make the experiment more critical the amount of serum inoculated into the mice eight hours

TABLE V.

No. of Guinea Pigs.	*Culture.*	*Interval.*	*Serum* 1931.	*Result.*
4	0·5 c.c. intramuscularly	Simultaneously	5 c.c. subcutaneously	D.L.L.L.
4	,, ,,	2 hrs. later	,, ,,	D.D.D.L.
4	,, ,,	4 ,, ,,	,, ,,	D.D.L.L.
4	,, ,,	———	None	D.D.D.D.
No. of Mice.				
4	0·3 c.c. subcutaneously	Simultaneously	1 c.c. intravenously	L.L.L.L.
4	,, ,,	2 hrs. later	,, ,,	L.L.L.L.
4	,, ,,	4 ,, ,,	,, ,,	L.L.L.L.
4	,, ,,	6 ,, ,,	,, ,,	L.L.L.L.
4	,, ,,	———	None	D.D.L.L.
4	0·5 c.c. ,,	8 hrs. later	0·5 c.c intravenously	D.D.D.L.
4	,, ,,	12 ,, ,,	1 c.c. ,,	D.L.L.L.
4	,, ,,	16 ,, ,,	,, ,,	D.D.D.L.
4	,, ,,	After symptoms had appeared.	,, ,,	D.D.D.D.
4	,, ,,	———	None	D.D.L.L.

after injection was reduced, but as some of these serum-treated animals were commencing to show symptoms of illness by the time the animals in the next group were due to receive serum a reversion was made to the larger quantity of serum.

The experimental work was next extended to sheep, the animal which we hoped might be protected by the inoculation of immune serum in the field.

Passive Immunity of Sheep.

As a preliminary experiment a sheep was inoculated with 20 c.c. of " ovine serum 1931 " and 48 hours later this sheep and a control were each inoculated intramuscularly with 0·5 c.c. of culture. The animal inoculated with serum remained well but the control sheep died from an acute gas gangrene infection within 16 hours.

Culture and serum were next inoculated simultaneously and in the first experiment " ovine serum 1931 " and the commercial serum were employed. The first portion of the experiment was designed in the hope of receiving from it data regarding the comparative value of the two sera. An infective inoculation of 0·5 c.c. of culture was used and against this large infective dose varying quantities of the two sera were tested.

Despite the very irregular results of this experiment, it is possible that some protection had been afforded to certain of the animals by the serum, as it was considered unlikely that any animal receiving 0·5 c.c. of the culture intramuscularly would have survived had serum not been given. But within the range of the amounts of serum given there appeared no optimum quantity and neither serum was clearly superior to the other.

TABLE VI.

No. of Sheep.	*Culture Inoculated Intramuscularly.*	*Serum.*	*Result.*
1	0·5 c.c.	50 c.c. ovine serum, 1931 simultaneously subcutaneously	Survived.
2	,,	20 c.c. ,,	Died 24 hrs. later.
3	,,	10 c.c. ,,	,, ,, ,, ,,
4	,,	10 c.c. ,,	Survived.
5	,,	50 c.c. commercial serum simultaneously subcutaneously	Chloroformed in extremis 48 hrs. later.
6	,,	20 c.c. ,,	Survived.
7	,,	10 c.c. ,,	Died 24 hrs. later.
8	,,	10 c.c. ,,	,, ,, ,, ,,
9	,,	None	,, 20 ,, ,,,
10	,,	,,	,, 40 ,, ,,

In the next step the test inoculation of culture was reduced in quantity and the experiment carried out in two parts. The value of a simultaneous subcutaneous inoculation of 20 c.c. of ovine serum 1931 was first tested, and as this gave encouraging results the experiment was repeated but augmented by a comparative test with the commercial serum.

TABLE VII.

1st Part. *Sheep.*	*Culture Inoculated Intramuscularly.*	*Serum.*	*Result.*
1	0·25 c.c.	20 c.c. ovine serum, 1931 simultaneously subcutaneously	Survived.
2	,,	,,	,,
3	,,	None	Found dead 42 hrs. later.
4	,,	,,	,, ,, ,, ,, ,,
2nd Part.			
5	,,	20 c.c. ovine serum, 1931 simultaneously subcutaneously	Died 4 days later.
6	,,	,,	Survived.
7	,,	20 c.c. commercial serum simultaneously subcutaneously	,,
8	,,	,, ,,	Died 21 hrs. later.
9	,,	None	,, ,, ,, ,,
10	,,	,,	,, 24 ,, ,,

From this experiment the value of the inoculation of ovine serum 1931 definitely appeared, three out of four sheep treated with serum surviving while all four control sheep died. Again, the fourth sheep inoculated with serum lived for a much longer period than did any of the controls. Of the two animals treated with commercial erum one survived.

The experiment was repeated with the infective inoculation of culture further reduced in amount. The same culture kept in cold store was used to furnish the infective inoculation in the experiments recorded on Tables VI, VII and VIII.

TABLE VIII.

Part I. *Sheep.*	*Culture Inoculated Intramuscularly.*	*Serum.*	*Result.*
1	0·0625 c.c.	20 c.c. ovine serum, 1931 simultaneously subcutaneously	Survived.
2	,,	,, ,,	,,
3	,,	None	Died 24 hrs. later.
4	,,	,,	Found dead 40 hrs. later.
Part 2.			
5	,,	20 c.c. ovine serum, 1931 simultaneously subcutaneously	Survived.
6	,,	,, ,,	,,
7	,,	20 c.c. commercial serum simultaneously subcutaneously	,,
8	,,	,, ,,	,,
9	,,	None	Died 21 hrs. later.
10	,,	,,	Survived.

From this experiment the value of the serum was again demonstrated, as all six sheep receiving serum lived and three out of the four controls died.

It was now considered that, provided the lethal dose of culture was not excessive, the serum had a decidedly favourable influence in overcoming the infection, for when the results of the experiments are grouped together they show that nine out of twelve sheep receiving " ovine serum 1931 " lived, whereas nine out of ten controls died.

In the comparative tests of the " ovine serum 1931 " and the commercial serum, five out of eight sheep treated with the former and four out of eight treated with the latter lived. As the results of the comparative experiments on sheep and guinea-pigs slightly favoured the use of the ovine serum, further experimental work was confined to the ovine sera.

The Effect of a Time Interval Between the Inoculation of Culture and the administration of Serum.

In the following experiment serum was inoculated four hours after the inoculation of culture and the experiment was carried out in two parts. In the first part the amount of culture inoculated was 0·25 c.c., but both animals receiving serum and the control animals died of gas gangrene, and in the second part where the culture was reduced to 0·0625 c.c. the animals receiving serum survived but so did one of the two controls.

TABLE IX.

Part 1. *Sheep.*	*Culture Inoculated Intramuscularly.*	*Serum.*	*Result.*
1	0·25 c.c.	20 c.c. ovine serum, 1931 simultaneously subcutaneously	Died in 72 hrs.
2	,,	,, ,,	,, ,, 28 ,,
3	,,	None	,, ,, 21 ,,
4	,,	,,	,, ,, 24 ,,
Part 2. 5	0·0625 c.c.	20 c.c. ovine serum, 1931 simultaneously subcutaneously	Survived.
6	,,	,, ,,	,,
7	,,	None	,,
8	,,	,,	Died in 21 hrs.

During the past lambing season a number of favourable results were reported from the fields when the administration of serum had been delayed until several hours after assistance had been given to ewes and the shepherd suspected them of having contracted a gas gangrene infection. Also the inoculation of serum into lambs already showing symptoms had apparently averted the disease. Comparatively large doses of serum had been inoculated into these ewes and lambs and in some cases two inoculations with an interval between had been made. Proof that the animals were infected with *B. chauvœi* was lacking, but it is likely that they were so infected because *B. chauvœi* infection had been diagnosed from the bacteriological examination of cadavers of incontact ewes which had not received serum and of two of the lambs which died shortly after inoculation with serum. The use of serum was not expected to give favourable results in lambs, as they would not be treated until symptoms were shown and the disease widely established. These favourable reports were obtained for the ovine serum drawn in 1932, for which our preliminary titration experiments indicated a high potency. This serum was therefore tested further for its capacity to neutralise or overcome an infection already established in the body. It will be seen from the Table X and the interpretation of the results that the subcutaneous or intravenous inoculation of 100 c.c. of serum given four hours after the inoculation of culture prevented the animals succumbing to a *B. chauvœi* infection and that the intravenous inoculation of large quantities of serum arrested the advanced symptoms of the disease and led to recovery.

The course of the infection in Sheep No. 2 was peculiar ; typical signs of an intramuscular inoculation infection with *B. chauvœi* were not seen, the inoculated limb showing neither tumefaction, œdema, nor discoloration. Between the eighteenth and thirty-sixth hours after the inoculation of culture the sheep remained lying down but did not show signs of acute pain. It was found dead in the morning of the third day and post-mortem examination revealed

TABLE X.

THE SAME CULTURE WAS USED FOR ALL THE INFECTIVE INOCULATIONS.

Sheep.	*Date of Inoculation.*	*Culture Inoculated Intra-muscularly.*	*Interval between Inoculation of Culture and Serum.*	*Serum.*	*Result.*
1	12.4.32	0·0625 c.c.	——	None	Died in 30 hrs.
2	13.4.32	,,	4 hrs.	100 c.c. subcutaneously	Died 3rd day. Atypical case, very mild local lesion but fœtus in uterus showed an acute infection with *B. chauvœi.*
3	14.4.32	,,	4 hrs.	100 c.c. intravenously.	Survived.
4	14.4.32	,,	,,	,, ,,	,,
5	15.4.32	,,	,,	100 c.c. subcutaneously.	,,
6	15.4.32	,,	——	None	Died in 21 hrs.
7	18.4.32	,,	8 hrs.	100 c.c. intravenously.	Survived.
8	18.4.32	,,	,,	,, ,,	,,
9	20.4.32	,,	12 hrs. when symptoms warranted a fatal prognosis had serum not been given.	2 intravenous inoculations each of 100 c.c. at intervals of 2 hrs.	Killed 5 days after infections owing to debility following the removal of a very decomposed immature fœtus 36 hrs. after infection; only traces of retrogressive *B. chauvœi* infection found at post-mortem.
10	20.4.32	,,	,, ,,	,, ,,	Survived.

only a very slight intramuscular and subcutaneous œdema of the inoculated thigh ; no muscle lesions were found. The animal, however was pregnant and the muscular and subcutaneous tissues of the intra-uterine fœtus showed extensive blackquarter or gas gangrene lesions. The integument was distended by gas and smears from the fœtal musculature showed very large numbers of bacteria morphologically identical with *B. chauvœi.* Only after prolonged search of smears made from the ewe's muscles at the seat of inoculation were micro-organisms recognised.

Sheep No. 9 was inoculated with serum twelve hours after the administration of an infective dose of culture and by this time it was registering a temperature of 105° F. and breathing was accelerated ; the inoculated limb was swollen and carried off the ground, and when disturbed the sheep fell and remained lying down on its side. At this time 100 c.c. of serum was given intravenously. Two hours later the temperature registered 106·4° F., and another inoculation of 100 c.c. of serum was made. By the sixteenth hour the temperature had dropped to 105·3° F. The animal was left for the night and

on the following morning, 36 hours after infection, a putrid discharge was found coming from the vulva. Examination showed a disintegrating, putrid, immature fœtus protruding into the vagina. This was removed in pieces. From the appearance of the fœtus it apparently had died some time before the commencement of the experiment. During the ensuing three days the ewe remained recumbent in an exceedingly weak condition, though eating and drinking when food and water were placed within reach. The condition of the animal remained stationary and on the fifth day it was considered advisable to slaughter her. At post-mortem examination the carcase was found to be poor and wasted, there was no involution of the uterus and this organ contained between 300 c.c. and 400 c.c. of thin, dark-coloured, putrid fluid. The inoculated limb was œdematous below the hock but elsewhere normal in size. Here and there in the depth of the thigh muscle were small dark areas or streaks—apparently all that remained of a gas gangrene or blackquarter muscle lesion. When thick smears were made from the dark coloured areas only one micro-organism was found per 15 to 20 fields of the microscope examined, and a growth of *B. chauvœi* was only obtained in minced meat broth medium when a piece of infected muscle the size of a filbert nut was inoculated. Where smaller pieces were inoculated no growth occurred, and a culture tube inoculated with 1·5 c.c. of the œdematous fluid from the lower part of the limb remained sterile.

Sheep No. 10 was lame and dull twelve hours after infection and registered a temperature of 102° F. Respirations were rapid and the animal allowed itself to be approached without moving. 100 c.c. of serum was inoculated at this time and again two hours later, when its temperature was 104° F. and the inoculated limb was very swollen and hot. By the 16th hour the temperature was 103° F., the sheep noticeably brighter, and the swelling of the limb felt cooler and less tense to the touch. Thirty-six hours after infection the animal appeared bright and normal except for its incapacitated leg, but by the eighth day only a slight limp remained and this eventually disappeared.

Had Sheep Nos. 2 and 9 not been pregnant they would in all probability have survived, as in both instances progressive local inoculation lesions had been arrested and recovery from these was occurring.

The experiments recorded in Table X are a testimony to the curative properties of immune serum and materially substantiate the claim that the inoculation of serum before or simultaneously with the contraction of infection is of prophylactic value.

Summary and Conclusions.

The great majority of cases of gas gangrene of sheep on the Romney Marsh are caused by *B. chauvœi*.

Parturient ewes suffer more than other sheep.

The disease is irregular in incidence and it is unlikely that active immunisation would be practised by owners, but prophylaxis by passive immunisation of individual ewes receiving assistance at lambing and thus exposed to infection would be undertaken. Accordingly experimental work has been concerned with passive immunisation. Experimental gas gangrene in sheep following upon intramuscular inoculation with cultures of *B. chauvœi* has been averted by the inoculation of hyperimmune serum prepared against ovine strains of *B. chauvœi* when the serum inoculated subcutaneously before, simultaneously with, or even four hours after the inoculation of culture, and by the intravenous inoculation of serum eight hours after infection. Further, when the inoculation disease has been allowed to progress until symptoms are acute and a fatal prognosis can be given from the symptoms shown, the disease may be overcome by the intravenous inoculation of large quantities of serum.

Preliminary experimental work was carried out upon guinea-pigs and mice, but final conclusions have been drawn by using sheep as the experimental animals.

These conclusions warrant the use of immune serum in the field on cases when there is reason to suspect that a *B. chauvœi* infection may be or may have been acquired.

H. R. GRUBB, LTD., CROYDON.

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A SPORULATING ANÆROBIC BACILLUS, SIMILAR TO THE CAUSAL ORGANISM OF BLACK DISEASE.

A. D. McEwen,
South-Eastern Agricultural College, Wye, Kent.

The bacillus described in this paper has been isolated in four instances from the tissues of sheep which had died suddenly under circumstances where pathogenic anærobic bacteria were suspected as the cause of death, although there was no history of recent parturition or of wounding.

It has been isolated once in over 150 examinations made upon the tissues of sheep from the Romney Marsh, Kent; but it has been encountered three times in a total of five examinations made upon material received from Wales—twice from the tissues of three sheep from North Wales and once from material from two sheep sent from South Wales.

These figures suggest a peculiar geographical distribution of the bacillus or that the susceptibility of sheep in different localities varies considerably.

In two instances the bacillus was isolated from heart blood and in the remaining two cases from voluntary muscle. In each case the material appeared to be fresh, and microscopical examination showed what might reasonably be considered pure cultures of a Gram-positive bacillus with rounded ends, occurring singly, in pairs, and in short chains or three to four individuals.

Each strain was purified by picking isolated colonies from shake agar cultures and sowing into minced meat medium, from which further shake agar cultures were made and colonies picked, and so on until it was assumed that the cultures were pure.

Morphology. Twenty-four hour cultures in broth containing minced meat gave a large, stout, Gram-positive and irregularly Gram-positive rods with rounded ends up to 12 to 14μ in length. A few bacilli stained irregularly with methylene blue, the organisms having a banded appearance. Occasional bacteria had a subterminal area of constriction. Spores were present in a number of instances. The fully developed spore was of greater breadth than the bacillus. Spindle and citron forms were absent.

Motility.

Motility has not been observed in sealed capillary tubes, or in slide and cover slip preparations sealed round with vasaline.

Cultural characteristics.

The bacillus is anærobic.

Broth containing minced meat.

Growth occurred in this medium with a very moderate production of gas. No colour change was produced in the meat, and the meat was not digested.

Broth.

Growth in medium was not abundant. At first a uniform haze appeared and after twenty-four hours this settled out as a light billowy cloud in the lower portion of the tube.

Milk.

No change was produced in this medium even after prolonged incubation.

Milk containing a portion of brain tissue.

At the end of a week's incubation a soft clot was formed and the expressed whey was clear. Later the clot gradually diminished in size and both clot and whey assumed a buffy tint. At the end of four weeks only a small clot was present in the bottom of the tube, digestion of the clot having occurred to a considerable extent.

Broth containing a portion of brain tissue and a piece of iron wire.

At the end of three days there was slight blackening of the brain and after one week's incubation this was very distinct. In the absence of iron wire no blackening occurred.

Gelatin containing a portion of brain tissue.

The gelatin was slowly liquefied.

Shake liver agar.

Colonies were readily obtained and the majority of these had a compact though irregular centre surrounded by a zone of openly woolly or filamentous growth. Light fluffy or woolly colonies with a compact centre were formed, as were colonies of compact growth surrounded by a number of pin point compact bodies. Simple lenticular colonies, and lenticular colonies with outcrops or tufts of growth form the centres of their biconvex surfaces, were also produced. Fragmentation of the media by gas only occurred when the colonies were very numerous.

Blood agar plates.

Fine delicate irregular colonies were grown ; these were slightly raised towards the centre and had delicate irregular filamentous processes radiating from them. They were surrounded by a zone of hæmolysis.

Serum agar.

Repeated attempts to grow the bacillus on 5, 10, and 30 per cent. serum agar have been made, but growth has only been obtained

in two instances from two of the strains on freshly prepared 10 per cent. serum agar. The colonies, except that they were smaller, resembled those produced by *B.œdematiens*. The serum used in the preparation of the media had been subjected to a temperature of 56° C. for two to three hours. Fresh unheated serum has not been tried. The heating of the serum may account for the absence of growth on serum agar.

No surface growth was formed on Fildes' influenza medium, liver agar, or liver glucose agar, although deep colonies grew readily in the two latter media.

Fermentation reactions.

Glucose, lactose, and maltose were fermented and after growth had ceased these sugars were not demonstrable by the Benedict qualitative method.

There was a doubtful or weak fermentation of glycerine, but the bacilli did not ferment lactose, saccharose, salicin, inulin, or mannite.

Pathogenicity.

0·1 c.c. of a broth culture was pathogenic for guinea-pigs; lesser amounts were not tested. In the inoculated animals an extensive œdema, almost colourless or faintly blood-tinged, was present in the subcutaneous tissues. The lesions were in all respects comparable with those produced by *B. œdematiens*.

Broth cultures of two of the strains were inoculated in amounts of 2 c.c. and 1 c.c. respectively into each of two sheep. In each case death followed in less than 24 hours. The post-mortem examinations were characterised by extensive œdema of the inoculated parts, considerable congestion of the mucosa of the fourth stomach and the small intestine, and in one instance hæmorrhage into the lumen of the bowel. Transudations of fluid were found in the thoracic cavity and the pericardial sac, the amount of fluid in the latter being particularly excessive. Bacteriological examinations failed to reveal the bacteria except in the tissues and fluid around the inoculated parts.

The intravenous inoculation of filtrates of broth cultures into rabbits caused death in two or more hours, depending upon the amount given and its toxicity. At autopsy extensive transudation of pale fluid were found in the thoracic cavity and the pericardial sac, 30 c.c. and 10 c.c. respectively. The lungs were œdematous and frothy. The lesions in these animals were indistinguishable from those produced by *B. œdematiens* toxin.

Immunity.

A formalinised culture of one strain was inoculated intravenously into two rabbits respectively in doses of 1 c.c., 1 c.c., 3 c.c. and 6 c.c. at intervals of two days. Ten days after the final inoculation one rabbit received 1 c.c. of toxic filtrate intravenously and the other 0·5 c.c. of filtrate intramuscularly. Both animals remained well,

but a control rabbit inoculated intravenously with 0·1 c.c. of filtrate died in 10 hours and another receiving 0·5 c.c. intramuscuarly was found dead five hours later. A very complete immunity had therefore been produced in the treated animals.

A few days later serum was collected from the immune rabbits and this serum when mixed *in vitro* with the toxic filtrate of the three heterologous strains and with filtrate of a strain of *B. œdematiens*, obtained from the National Collection of Type Cultures, caused in each instance complete neutralisation of the toxin, a neutralisation not produced by normal rabbit serum. Further, *B. œdematiens* antiserum, kindly given to me by Mr. T. Dalling, of the Wellcome Physiological Research Laboratories, Beckenham, when mixed *in vitro* with the toxic filtrates of the ovine strains of bacteria caused neutralisation of the toxin.

Discussion.

The four strains of the bacillus which have been described show similarities and differences from *B. œdematiens*. In pathogenicity they are alike and the toxin of each is neutralised by the immune serum of the other, but morphologically and culturally distinctions exist which prevent the bacteria being regarded as identical.

The more distinct cultural differences are tabulated below. These may be summarised by saying that the ovine strains studied were less easily cultivated but more proteolytic than typical *B. œdematiens*. Further the ovine strains are distinctly the larger of the two.

	Ovine Strains.	*B. oedamatiens.*
Serum agar slants	Growth not obtained or only obtained with difficulty	Growth easily obtained
Milk	No growth	Growth
Milk plus brain tissue	Casein digested	Casein not digested
Broth containing brain tissue and iron wire	Brain blackened	Brain not blackened

A consideration of the descriptions by Turner & Davesnes (1927) and Turner (1930) of the bacillus of Black Disease reveals much that is common to that organism and the bacilli isolated from sheep in this country.

Thus they both present similar difficulties in cultivation and are alike in size and morphology. Both show a similar range of deep colony types in shake agar and comparable surface colonies on blood agar. They each digest casein and blacken brain in the presence of iron, and ferment glucose, lævulose, and maltose. Their toxins so far have not been distinguished from the toxin of *B. œdematiens*.

Slight differences, however, are to be noted. The strains examined in this laboratory were not observed to be motile, whereas Turner (1930) describes the Black Disease bacillus as sluggishly motile, although motility was not observed by Turner and Davesnes

(1927). Further, the surface colonies of the Black Disease bacillus are not hæmolytic on blood agar, although the toxin is hæmolytic for sheep's erythrocytes *in vitro* (Turner, 1930), but the strains from this country were hæmolytic on blood agar medium.

These differences, however, may be attributable to variation in individual technique and interpretations, and it would appear probable that the strains isolated in this country and the bacillus of Black Disease are like variants of *B. œdematiens*.

At present Black Disease is only reported from Australia and New Zealand, and possibly Tasmania, and it is believed that the presence of immature liver fluke in the hepatic tissues function as activators for the spores of the bacillus lying latent in these tissues and that after activation localised areas of bacterial activity give rise to necrotic hepatitis, toxæmia, and death.

In view of the prevalence of liver fluke disease it may be reasonable to suppose that outside certain districts in the aforementioned countries the spores of the causal micro-organism of Black Disease are not present in a quiescent state in the livers of sheep, or that Black Disease, if it occurs in other parts of the world, passes without recognition.

Summary.

From material received from a total of five sheep which came from Wales a bacillus similar to the *B. œdematiens* type of micro-organism which is held responsible for Black Disease has been isolated in three instances. The same bacillus has been isolated in one instance only from material from over 150 sheep from the Romney Marsh, Kent.

These findings suggest a peculiar local distribution of the micro-organisms. If such be the case, does Black Disease occur in this country in districts where the liver fluke and the *B. œdematiens* type of bacillus occur, or does this micro-organism cause disease in sheep independently of any liver fluke disease ?

References.

Turner, A. W., and Davesnes, J. 1927. Ann. Inst. Pasteur, 41, 1078.

Turner, A. W. 1930. Bul. No. 46. Council for Scientific & Industrial Research, Australia.

Note.—Since the above paper was written an article by H. Miessner, A. Meyn, and G. Shoop, Centralt. f. Bakt., 1931, 120, p. 257, has been brought to my notice. In this article bacilli of the *B. œdematiens* type, and probably identical with the bacillus of Black Disease, are considered to be the cause of Bradsot in certain districts in Germany. In cases examined before putrefactive changes were advanced necrotic areas were found in the liver. These were regarded as similar to the liver lesions in Black Disease. However, no co-relation between liver fluke infestation and Bradsot was established, although some of the animals harboured the parasites.

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